

Vol. 32, No. 3

August 1945
New York: 1945

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

R. W. MARSH

WITH THE ASSISTANCE OF

F. C. BAWDEN

H. MARTIN

J. W. EVANS

I. THOMAS



CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)

BOMBAY, CALCUTTA, MADRAS: Macmillan

All rights reserved

PRINTED IN GREAT BRITAIN

The Association of Applied Biologists

President: C. B. WILLIAMS, M.A., Sc.D.

Vice-Presidents: PROF. W. BROWN, M.A., D.Sc., F.R.S. AND W. C. MOORE, M.A.

Hon. Treasurer

W. C. MOORE, M.A.
Plant Pathology Laboratory,
Milton Road, Harpenden, Herts.

Hon. Editor

PROF. W. B. BRIERLEY, D.Sc.
University of Reading,
Berk.

Hon. Assistant Editor

R. W. MARSH, M.A.
Research Station,
Long Ashton, Bristol.

Hon. Secretary

W. P. K. FINDLAY, D.Sc.
Forest Products Research Laboratory,
Princes Risborough, Bucks.

Hon. Assistant Secretary

H. SHAW, M.Sc., Ph.D.
East Malling Research Station,
Nr. Maidstone, Kent.

Council

L. A. ALLEN, D.Sc.
G. M. BATES, D.Sc.
F. C. BAWDEN, M.A.
J. R. BUSVINE, Ph.D.

M. COHEN, Ph.D.
G. V. B. HERFORD, B.A.
J. T. MARTIN, D.Sc.
E. W. MASON, M.A.

H. W. MILES, D.Sc.
M. H. MOORE, M.Sc.
R. T. PEARL, B.Sc.
MISS A. P. WILSON, A.R.C.S.

Plant Pests and Diseases Committee

Hon. Secretary: W. G. KEYWORTH, Ph.D., A.R.C.S., East Malling Research Station,
Nr. Maidstone, Kent.

CONTENTS OF VOL. 32, No. 3

	PAGE
1. The parasitism of <i>Striga hermonthica</i> Benth. on <i>Sorghum</i> spp. under irrigation. I. Preliminary results and the effect of heavy and light irrigation on <i>Striga</i> attack. By F. W. ANDREWS. (With 1 Text-figure)	193
2. The influence of date of lifting and method of storing on loss of onion bulbs harvested in 1943. By E. R. WALLACE and C. J. HICKMAN. (With 1 Text-figure)	200
3. Black scurf and stem canker of potato (<i>Corticium Solani</i> Bourd. & Galz.). Further field studies on the use of clean and contaminated seed potatoes and on the contamination of crop tubers. By T. SMALL, Ph.D., A.R.C.S.	206
4. Experiments on the control of white rot (<i>Sclerotium cepivorum</i> Berk.) in onions. By J. R. BOOER, Ph.D., B.Sc. (With 1 Text-figure)	210
5. Gliotoxin, a fungistatic metabolic product of <i>Trichoderma viride</i> . By P. W. BRIAN and H. G. HEMMING. (With 1 Text-figure)	214
6. Studies in the dynamics of disinfection. IV. The reaction between phenol and <i>Bact. coli</i> : the true shape of the probit-log survival-time curve. By R. C. JORDAN, Ph.D. and S. E. JACOBS, Ph.D. (With 7 Text-figures)	221
7. The use of abrasives in the transmission of plant viruses. By H. KALMUS and B. KASSANIS. (With Plate 8)	230
8. Changes in wireworm population associated with cropping. By H. W. MILES and M. MILES	235
9. Control of the potato-root eelworm, <i>Heterodera rostochiensis</i> Wollenweber, by allyl isothiocyanate, the mustard oil of <i>Brassica nigra</i> L. By C. ELLENBY, B.Sc., Ph.D.	237
10. Studies upon the copper fungicides. VII. The solution of copper from dressings on the pea seed. By R. L. WAIN and E. H. WILKINSON	240
11. Studies upon the copper fungicides. VIII. The penetration of copper into germinating peas. By R. L. WAIN and E. H. WILKINSON	243
12. The mode of action of sheep dips. By E. T. BURTT, M.A., Ph.D.	247
13. Proceedings of the Association of Applied Biologists: 8 December 1944	
Bloat or eelworm disease of onions: recent investigations. By T. GOODEY, D.Sc.	261
Symposium on the carrot fly:	
1. The biology and control of the carrot fly. By F. R. PETHERBRIDGE, M.A., Dip. Agric. (Cantab.), D. W. WRIGHT, M.A. and D. G. ASHBY, B.A. (With 1 Text-figure)	262
2. The carrot fly in the Midlands. By A. ROEBUCK	264
3. Investigations on the control of carrot fly (<i>Psila rosae</i> F.) in gardens. By G. FOX WILSON. (With 7 Text-figures)	265
14. Proceedings of the Association of Applied Biologists: 23 March 1945. Symposium on the genetical relations of plants and animals to their pests and diseases:	
Introduction: the genetic analysis of disease. By C. D. DARLINGTON	277
'Constitution' in cattle in relation to pests and diseases. By J. HAMMOND	278
Observations on heritable disease in poultry. By M. S. PEASE	279
Heritable susceptibility of poultry to cancer virus. By J. G. CARR	279
Inheritance of resistance to blight in potatoes. By W. BLACK	279
Some genetical aspects of resistance to potato viruses. By G. COCKERHAM	280
Diseases and pests at the Welsh Plant Breeding Station, Aberystwyth. By T. J. JENKIN	281
General discussion and summing up. By C. B. WILLIAMS	281
15. Reviews	283

The parasitism of *Striga hermonthica* Benth. on *Sorghum* spp. under irrigation

I. Preliminary results and the effect of heavy and light irrigation on *Striga* attack

By F. W. ANDREWS, *Senior Economic Botanist, Research Division, Department of Agriculture
and Forests, Wad Medani, A.E. Sudan*

(With 1 Text-figure)

Investigations were carried out at the Gezira Research Farm in the Anglo-Egyptian Sudan. The greater majority of the seeds of *Striga hermonthica* can only be germinated by excretions from roots of certain plants not all of which can act as hosts for this parasite. Unless it becomes attached to a host plant, the *Striga* seedling dies. The growing of those plants whose roots can stimulate the germination of *Striga* seed but cannot be parasitized by it, may be a means of ridding infested land of this parasite. Green ovaries picked from flowering plants produce viable seeds if left to dry. Sorghum is sown in the field during the rainy season, viz. June to October: the earlier the sowing date within this period the greater is the *Striga* attack. In the field *Striga* seeds are distributed in the soil to a depth of at least 15 in. When *Striga* seeds are evenly distributed through the soil, the number of *Striga* seedlings attached to a sorghum root is proportional to the root's development. Size of sorghum seed has no effect on the root size of a sorghum plant and consequently no effect on the degree of parasitism. The effect of severe *Striga* attack on the sorghum plant is to produce a reduction of about 60% in leaf and root weight. No reduction of *Striga* attack is obtained when various micro-elements are coated on sorghum seeds before sowing. Field and laboratory experiments show that light irrigation of the sorghum crop during the normal sowing period increases the *Striga* attack and heavy irrigation decreases it. This result was not obtained in laboratory experiments when sorghum was sown out of season. *Striga* attack is lessened when conditions favouring growth of the sorghum crop are improved.

INTRODUCTION

These investigations were carried out in the Anglo-Egyptian Sudan at the Gezira Research Farm which lies towards the southern part of the large irrigated cotton scheme worked as a concession by the Sudan Plantation Syndicate. The soil of the Research Farm is a heavy clay, strongly alkaline (pH up to 9.4), of low permeability to water and of low content of nitrogen and humus (Joseph, 1925).

Sorghum forms a part of the prevailing rotation on this land, and in the earlier years of the concession, when a three-course rotation was practised, *Striga* infestation was often severe. On a part of the Research Farm a three-course rotation is still in operation, and the ravages of *Striga* on sorghum plots are frequently heavy and have on occasions completely vitiated the results of experiments.

THE LIFE HISTORY OF *STRIGA HERMONTICA* BENTH.

Striga hermonthica Benth. occurs throughout the tropics and subtropics of the Old World. Among crops it has been recorded as affecting the yields of sorghum, sugar cane, and maize. It is a partial

parasite on the roots of these crops, and under severe infestation the yield of the crop may be reduced to nil and the host plants killed.

The life history of a near relative (*S. asiatica* (L.) D. Kuntze = *S. lutea* Lour.) of this plant has been studied in detail by Saunders (1933), and the structure and development of the haustorium were described by Stephens (1912). *S. asiatica* is similarly parasitic on sorghum and maize. The mode of parasitism and development of *S. hermonthica* differs little from that of *S. asiatica*. The former plant shows the characteristics of the Scrophulariaceae and is an erect, usually branched, green, hispid herb up to 2 ft. high, but more usually about 15 in. The conspicuous flowers vary greatly in colour from rose red (the commonest colour) to the more rare pale pink-white. On an average there are about 60 capsules per plant, each of which contains about 700 dust-like seeds: thus, on average, one plant produces about 42,000 seeds. The seeds are dark brown in colour and concentrically and reticulately ridged. The plant dries to a dark blue colour in common with other parasitic members of the family. Germination of the majority of the seeds is brought about under the

stimulus of excretion from the roots of certain plants. The plants whose root excretions cause germination of the *Striga* seeds are not necessarily parasitized by the *Striga* plant.

Experiments were made to test the germinating power of the root excretions of different plants on the seeds of this parasite. The technique used was as follows: the bottom of a half Petri dish of 11 cm. diam. and 3 cm. height was covered with blotting paper strewn with *Striga* seeds, so that the seeds lay against the glass. Through holes pierced in the blotting paper seedlings of the different plants (one species to each dish) were arranged so that the root lay between the blotting paper and the glass, the hypocotyl and cotyledons standing vertical. The dish was nearly filled with clean sand and watered. The dishes with their seedlings were kept in a closed glass-sided chamber, the floor of which was covered with sand kept permanently moist to ensure that the plants grew in an atmosphere of high humidity. The plants grew satisfactorily, and the roots developed among the *Striga* seeds. Periodic observations through the glass bottom showed the course of development of the root and the degree of germination of the *Striga* seeds. Controls consisted of similarly prepared dishes without seedlings. Unless the germination in the dish under test was much greater than that in the control (which in most cases was nil) the plant was not considered to have roots which could stimulate the germination of *Striga* seeds.

A list of plants whose roots have been observed by the author to cause germination is given. Except for some of the grasses and *Cassia ashrek* the plants tested are crop plants in the Sudan.

It will be noted that the dominant host plants of this parasite are grasses. With the exception of *Dolichos Lablab*, none of the non-gramineous plants is parasitized. It is likely that *Striga* seeds germinated in the absence of gramineous host plants will die, since, as far as is known, attachment to a host plant is necessary for the further development of the *Striga* seedling. Parasitism on *Dolichos Lablab* has only been noticed in pots, never in the field, and it is possible that germination in the field by these non-gramineous plants is not so prolific as under laboratory conditions. Nevertheless, the fact that it does occur without parasitism of the stimulating root demonstrates important possibilities of control of this parasite.

These tests showed some characteristics of the germination of *Striga* seed, certain of which have also been noted by Doughty (1942). Only a very small proportion of the seeds germinate, the germination being confined to such seeds as are in the immediate neighbourhood of the activating root. The seeds remain ungerminated at a distance greater than 1 cm. from the plant root. Germination occurs after 3 days from the date of insertion of the plant root. The radicle does not necessarily grow in the direction

of the activating root. On reaching the root of a plant that can be parasitized, the radicle forms a swelling at the junction, the intervening tissue between host root and parasite root is dissolved and the conducting tissue of both host and parasite becomes united. Only when this union is effected does the *Striga* seedling begin to develop its plumule, the precursor of its aerial growth.

It has been demonstrated that seeds can remain viable in the soil of the Gezira Research Farm for 5 years, but it is highly probable that they will still germinate after a much longer period of dormancy.

Plants whose root excretions can germinate the seeds of Striga hermonthica Benth.

LEGUMINOSAE

*† <i>Dolichos Lablab</i> L.	...	...	bonavist bean
<i>Vigna unguiculata</i> Walp.	...	...	cowpea
† <i>Arachis hypogaea</i> L.	...	...	groundnut
<i>Cassia ashrek</i> Forsk.	...	...	Italian senna
<i>Cicer arietinum</i> L.	...	...	chick pea
<i>Cajanus Cajan</i> Millsp.	...	...	pigeon pea
† <i>Phaseolus vulgaris</i> L.	...	...	French bean
<i>Lupinus termis</i> Forsk.	...	...	lupin
<i>Vicia faba</i> L.	...	...	broad bean
<i>Glycine max</i> (L.) Merr.	...	...	soya bean
<i>Pisum sativum</i> L.	...	...	peas
<i>Medicago sativa</i> L.	...	...	lucerne
† <i>Crotalaria juncea</i> L.	...	...	sunhemp

GRAMINEAE

*† <i>Sorghum</i> spp.	...	...	millet
*† <i>Pennisetum</i> spp.	...	...	bulrush millet
* <i>Paspalum commersonii</i> Lam.	...	...	
* <i>Panicum miliaceum</i> L.	...	...	French millet
* <i>Panicum miliare</i> Lam.	...	...	little millet
* <i>Oryza</i> spp.	...	...	rice
* <i>Cynodon Dactylon</i> Pers.	...	...	Bermuda grass
* <i>Setaria italica</i> Beauv.	...	...	Italian millet
* <i>Euchlaena mexicana</i> Schrad.	...	...	teosinte
*† <i>Zea Mays</i> L.	...	...	maize

OTHER FAMILIES

<i>Gossypium barbadense</i> L.	cotton	Malvaceae
<i>Sesamum orientale</i> L.	simsim	Pedaliaceae
† <i>Helianthus annuus</i> L.	sunflower	Compositae
<i>Cucumis Melo</i> L.	melon	Cucurbitaceae

* Parasitized by *S. hermonthica*.

† Shown by Rose & Lochrie (1941) to germinate seed of *S. asiatica*.

Seeds formed from immature ovaries

The only method of control for this parasite adopted on the Research Farm is to pull the plant by hand before it flowers. It was found early in these investigations that the green ovaries in the flowering plant produce ripe seeds if they are left to lie on the ground. It is therefore necessary to destroy immediately any flowering plants that have been pulled from the sorghum before they can produce ripe seeds. All laboratory experiments described in this paper were performed with *Striga* seeds produced

from picked green ovaries. Spraying the *Striga* plants in the field with up to 10% sulphuric acid proved ineffective; the aerial part of the *Striga* plant was generally killed by the acid spray, but the underground portion remained alive and later produced fresh aerial growth.

The relation of sowing date of sorghum and Striga attack in the field

The intensity of *Striga* attack was found to be intimately correlated with the sowing date of the sorghum crop, heavier *Striga* intensity being found

of *Striga* on the sorghum is, within limits, progressive, the earlier sowing would of necessity show more *Striga*. Experiments have, however, shown that *Striga* makes its appearance in the field at the end of the first month after sowing, reaches maximum flowering towards the end of the second month, and begins to die down towards the end of the third month. It is therefore possible that the infestation on the earlier sowing was even heavier than indicated. A similar sowing-date experiment was carried out in 1938 and the *Striga* infestation was estimated on 6 Oct. 1938 in a similar manner (see Table 2).

TABLE 1. *Relation of sowing date of sorghum to Striga attack in the field, 1935*

Month ...	Sowing date								
	June			July			Aug.		
Day ...	1	10	20	1	10	20	1	10	
Striga severity in 5 replicates	4	2	5	2	3	1	0	1	
	5	3	4	3	3	1	2	0	
	4	4	4	3	3	1	0	1	
	4	5	3	2	1	2	0	1	
	5	3	1	2	1	2	0	1	
Total	22	17	17	12	11	7	2	4	

in the earlier sown sorghum. This was clearly shown in a sorghum sowing-date experiment carried out on the Gezira Research Farm in 1935. Sorghum is sown in the rainy season, on ridges, with a spacing of 50 cm. between holes, and is watered when necessary by irrigation. There were eight sowing dates with five replicates of each. All subplots were examined on 4 Nov. 1935 for *Striga* plants, and the intensity estimated by eye on a 0-7 scale, 0 meaning no *Striga* plants, and 7 that the subplot was completely covered with *Striga* (see Table 1). It is well known that the number of aerial appearances of *Striga* is not necessarily the number of the *Striga* plants parasitizing a particular plant, since *Striga* plants on the host roots may exist below the soil surface. It has, however, been assumed in all field and some laboratory experiments that the aerial appearances provide a reasonable estimate of the total number of parasites infesting a particular plant.

It will be noted that there is a progressive reduction in total *Striga* intensity as the sowing date becomes later. It might be suggested that as the appearance

TABLE 2. *Relation of sowing date of sorghum to Striga attack in the field, 1938*

Month ...	Sowing date							
	June		July			Aug.		
Day ...	20	30	10	20	30		9	
Striga severity X2 in 5 replicates	8	10	3	3	1	—	—	
	11	5	3	1	1	—	—	
	11	4	1	2	1	—	—	
	9	7	1	4	1	—	—	
	8	2	0	1	1	—	—	
Total	47	28	8	11	5	—	—	

It is clear that the earliness of the sowing date of sorghum governs the subsequent *Striga* intensity.

The distribution of Striga seeds in the soil

Experiments were performed to determine the distribution of *Striga* seeds in the soil in the field. Soil was collected from different depths in five small areas in two 5-acre plots (nos. 30 and 45) which had previously carried a heavy infestation of *Striga*. Pots were filled with the soil (seven pots for each depth in each plot) and on 11 July 1942 were sown with five sorghum seeds per pot, later thinned to four. Table 3 gives the total number of *Striga* plants that appeared in the pots at each level.

In 1943 a similar experiment was performed using ten pots per depth in different areas in the same two plots (see Table 4).

In Table 3 there are more *Striga* seeds in the upper layers than in the lower layers: this is not so obvious in Table 4. It is clear, however, that any control measure involving treatment of the surface soil is not likely to be effective.

TABLE 3. *Distribution of Striga seeds in the soil, 1942*

Depth of soil in.	Plot 30 Area no.						Plot 45 Area no.					
	1	2	3	4	5	Total	1	2	3	4	5	Total
0-3	47	48	9	2	10	116	74	28	33	17	2	154
3-6	32	16	26	26	17	117	43	16	12	14	15	100
6-9	11	15	19	36	18	99	28	27	20	9	6	90
9-15	18	14	20	22	10	84	13	24	5	10	11	63

TABLE 4. Distribution of *Striga* seeds in the soil, 1943

Depth of soil in.	Plot 30 Area no.						Plot 45 Area no.					
	1	2	3	4	5	Total	1	2	3	4	5	Total
0-3	3	6	4	6	2	21	5	8	9	5	13	40
3-6	10	8	4	9	3	34	5	7	7	9	4	32
6-9	11	14	4	11	7	47	2	10	10	8	5	35
9-15	14	7	6	9	6	42	4	7	8	4	0	23

The rate of parasitism of the sorghum seedling

It was of interest to discover how soon after the sowing of the sorghum seed the *Striga* seedlings could be found parasitizing the sorghum roots. For this purpose eighty pots of 4 in. diam. were filled with soil that had been heated to 120°C. to ensure that it contained no viable *Striga* seeds. The soil of each pot was mixed with 0.1 g. of *Striga* seeds, and the pots were each sown with one sorghum seed which had been soaked for 24 hr. Ten pots were washed out every 7 days and the number of *Striga* seedlings attached to the roots was counted. Table 5 gives the mean number of *Striga* seedlings attached to the roots of a sorghum plant after every 7 days.

TABLE 5. Rate of parasitism of the sorghum seedling

Days after sowing	7	14	21	28	35	42	49
Mean no. of <i>Striga</i> plants	24	93	399	482	556	341	363

It was found in an experiment on the rate of germination of sorghum seeds in pots that it was not until 3 days after sowing that any radicle reached 18 mm. in length: after the fourth day lengths of 46, 35, 65 and 59 mm. were obtained. Since the sorghum roots were already parasitized 7 days after sowing, the germination of the *Striga* seed and subsequent parasitism of the sorghum roots must occur with great rapidity. Fig. 1 shows the average number of *Striga* seedlings per sorghum plant every 7 days. It will be noticed that the largest increase occurs between the second and third weeks, and after the fourth week there is little increase, while a decrease occurs after the fifth week. No *Striga* plants appeared above the soil surface during the second and third weeks, though by the end of the fourth week many *Striga* plants were very near to the surface. From this and other experiments it has been assumed that the bulk of the *Striga* attack occurs within the first 4 weeks after sowing, and that the *Striga* plants that appear above the ground are a visible expression of an attack which occurred much earlier in the sorghum plant's development. In Fig. 1 the curve up to the period of 5 weeks resembles a sigmoid growth curve. The logarithms of the values for 7, 14 and 21 days form a straight line showing that they lie on an

exponential curve, which is a characteristic of the earlier values of a growth curve. It is reasonable to assume, therefore, that in this experiment the number of *Striga* plants infesting a sorghum plant was proportional to some growth value, presumably the root development of the sorghum. The volume of soil in each pot was roughly the same, and the *Striga* seeds were as far as possible evenly distributed through the soil. It is clear, therefore, that with equal volumes

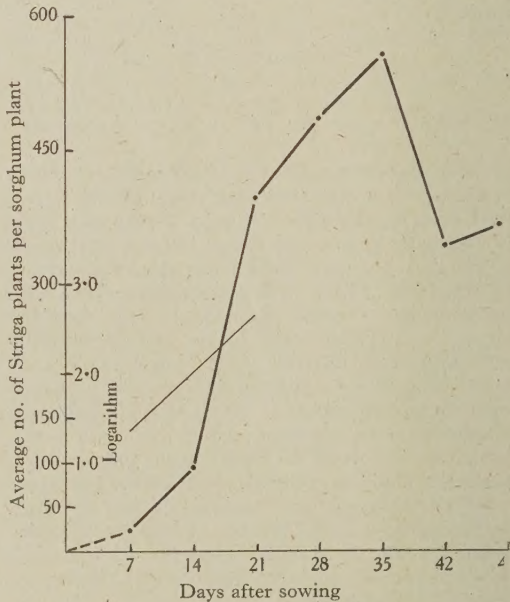


Fig. 1.

of soil and even distribution through the soil of the same quantity of *Striga* seeds the number of *Striga* plants attacking a sorghum root will be proportional to its development. The decrease in *Striga* number observed after the 35-day period may be due either to the roots of the sorghum becoming pot bound or to the fact that at this stage of the growth of the sorghum plants the root system contains both thickened and thin roots. The thickening of some of the roots may have caused some of the *Striga* seedlings to be shed.

*The Striga attack on sorghum seedlings
from seeds of different sizes*

It was thought that sorghum plants from small seeds might be less vigorous than those from larger seeds and possibly more susceptible to Striga attack, or that the root development would be greater from the larger seeds and consequently a single plant might sustain more Striga seedlings.

Sorghum seed was divided as follows:

Size no.	Wt. of 30 seeds g.
1	0.7216
2	0.9818
3	1.1216
4	1.2822

Forty 4 in. pots (= 10 replicates of each seed size) were filled with soil heated to 120°C. In each pot was mixed 0.15 g. of Striga seed. The pots were sown, one seed per pot, on 7 Sept. and the plants washed out on 4 Oct. 1943. The number of Striga seedlings attached to the roots of each plant was counted and the roots were then dry weighed. Table 6 gives the mean values per plant obtained in this experiment.

TABLE 6. *Striga attack on sorghum seedlings
from seeds of different sizes*

Seed size	Striga plants	Root wt. (g.)
1	265	0.0727
2	303	0.0747
3	265	0.0797
4	290	0.1009

An analysis of variance of the results showed that the mean differences in total Striga and root weight per seed type were not significant. It is thus clear that the size of seed has no effect on the degree of parasitism of a sorghum plant. A regression coefficient calculated between the number of Striga seedlings per plant and the root weight per plant was highly significant, showing that the number of seedlings parasitizing the sorghum plants was proportional to their root development.

*The effect of the Striga attack on the
sorghum plant*

The ravages of a Striga attack on sorghum are very obvious in the field. Stunted plants produce little or no grain; yellow leaves often dry and fall off, showing that the plants are being robbed of a major portion of their food supply. In a pot experiment an attempt was made to discover how much loss of plant material occurs when sorghum is severely attacked by Striga. Five pots (14 in. diam.) were filled with soil, and 0.4 g. of Striga seeds was mixed with the top 3 in. of soil. Five similar pots were prepared without the addition of Striga seeds. The pots were each sown with two sorghum seeds on 13 May 1942

and thinned to one plant per pot 5 days later. After 6 weeks' growth the plants were washed out. The number of Striga plants that had appeared above ground and also those that were attached to the roots but were still below the soil surface were counted, and the leaves and roots of the sorghum were dry weighed (Table 7).

The difference in root weight and leaf weight between parasitized and non-parasitized sorghum plants is highly significant. Striga has caused a reduction of 58% in leaf weight and 52% in root weight. A similar experiment yielded the results given in Table 8.

TABLE 7. *Effect of Striga attack on the sorghum
plant. Experiment 1*

Plant no.	With Striga				Without Striga	
	No. of Striga plants	No. of under- ground Striga plants	Root wt. g.	Leaf wt. g.	Root wt. g.	Leaf wt. g.
1	22	208	1.37	1.07	3.24	2.39
2	—	218	0.88	0.69	2.75	2.67
3	6	362	1.88	1.10	2.37	2.25
4	12	233	1.21	0.88	1.73	1.20
5	17	268	1.23	0.93	3.55	2.66
Total	57	1289	6.57	4.67	13.64	11.17

TABLE 8. *Effect of Striga attack on the sorghum
plant. Experiment 2*

Plant no.	With Striga				Without Striga	
	No. of Striga plants	No. of under- ground Striga plants	Root wt. g.	Leaf wt. g.	Root wt. g.	Leaf wt. g.
1	15	351	3.70	3.13	7.10	6.80
2	16	173	1.95	1.98	11.45	10.94
3	14	287	1.02	0.93	7.16	5.98
4	15	276	3.89	3.42	5.38	5.16
5	22	312	2.22	2.23	7.46	6.83
Total	82	1399	12.78	11.69	38.55	35.71

The differences in root and leaf weight between parasitized and non-parasitized sorghum plants are highly significant. Striga has caused a loss of 67% in both root and leaf weight. In both experiments the variation within treatments of leaf and root weights was great owing to the unavoidable use of impure sorghum seed. The results parallel the devastating effect of this parasite noted in the field.

It will be observed from Tables 7 and 8 that after 6 weeks' growth the average number of underground parasites attached to a sorghum root are roughly 22 and 17 times respectively the average number of Striga plants appearing above ground. The number of aerial Striga plants, while providing a useful estimate of the degree of parasitism, does not give a

true picture of the total parasitism under which the sorghum plant is growing. It seems apparent that many of these parasites do not reach the aerial stage, but remain as underground seedlings attached to the sorghum roots.

*The effect of coating sorghum seeds
with mineral salts*

The area under sorghum in the present Gezira irrigation scheme averages annually about 100,000 acres. In this large area any Striga control measure involving treatment of the soil to kill the Striga seeds lying in it is likely to be impracticable. Further, sorghum is a low-priced crop, and it is doubtful if it would sustain the cost of any such treatment. It was not known if the soil was lacking in some necessary micro-element, the addition of which might enable the sorghum plant to resist the attack of Striga at least in its early stages. The following experiment was carried out: 57 g. of sorghum seed was mixed with 1 g. of the finely powdered chemical + 1 g. of powdered gum acacia; the whole was shaken, and

TABLE 9. *Effect of coating sorghum seeds
with mineral salts*

Treatment	Total no. Striga plants
Borax	299
Copper sulphate	409
Manganese sulphate	320
Zinc sulphate	439
Gum only	315
Control (no treatment)	219

5 c.c. of water added; the whole was again thoroughly shaken and the seeds allowed to dry in the laboratory. By this means the seeds were coated with a layer of the chemical. The chemicals tested were borax, copper sulphate, manganese sulphate, zinc sulphate, with controls of gum only and untreated seed. The seed was sown on 1 July 1942 in soil in petrol tins, six seeds per tin, later thinned to four. The soil had previously been mixed with 0.4 g. of Striga seed per tin and there were ten replicates of each treatment. The total number of Striga plants per tin that had appeared above ground up to 27 Aug. 1942 was counted (see Table 9).

None of the differences in total Striga is significant. There was great variation in the total Striga plants per tin within treatments. It does not appear that micro-elements added to the soil by this method exercise any control over the Striga attack.

*The effect of light and heavy irrigation of the
sorghum crop on Striga attack*

The sorghum in the Gezira irrigation scheme is planted towards the end of July in areas roughly 1350 m. long by 280 m. broad. This rectangular area is watered by gravitational flow from a channel running along the length of the rectangle. In con-

sequence, the water tends to collect at the base of the rectangle, while the top of the rectangle is likely to be less heavily watered. It was noticed that the visible Striga attack was generally greatest at the top of the rectangle and least at the base. It appeared that the cause of this difference in intensity of Striga attack was differential watering. Under irrigation the watering rate is controllable, and it was thought that a measure of control of Striga might be obtained if, in fact, the Striga intensity depended on the amount of water the crop received. A field experiment was therefore designed to ascertain if this were true.

A 5-acre plot (no. 45) on the Gezira Research Farm was sown with sorghum on 1 June 1939. The plot was divided in thirty subplots, and on 28 Sept. the Striga per subplot was estimated by eye by two

TABLE 10. *Effect of light and heavy irrigation of
the sorghum crop on Striga attack*

Light-watering subplots			Heavy-watering subplots		
Sub-plot	1939 estimate	1940 increase or decrease	Sub-plot	1939 estimate	1940 increase or decrease
3	1.00	-0.25	1	1.25	+0.50
4	2.50	+3.00	2	1.25	-0.50
5	1.00	0.0	6	1.25	-0.50
7	2.00	+2.50	8	1.50	-0.50
11	1.50	+0.25	9	1.00	-0.50
12	1.00	0.0	10	2.50	+0.25
14	1.25	-0.25	13	1.75	0.0
15	1.00	0.0	17	1.00	+0.50
16	2.00	+1.25	18	1.00	-0.25
21	1.00	+0.50	19	2.00	0.0
22	3.00	+1.25	20	1.00	-0.25
23	1.00	+1.00	24	1.00	-0.25
25	3.00	+0.75	26	1.50	-0.25
27	1.50	+0.50	28	2.00	+1.75
29	1.25	+0.25	30	1.00	+0.25
Total	24.00	+10.75		21.00	+0.25

independent observers on a 0-7 scale, 0 meaning no Striga, and 7 that the whole subplot was covered with Striga. The average of the two estimates was taken as the correct value. In the following season the thirty subplots were sown again with sorghum on 15 June 1940. Fifteen of the subplots chosen at random were watered sufficiently to keep the sorghum alive, roughly about once a fortnight, and fifteen subplots were given weekly watering. The Striga infestation was again estimated by eye by two independent observers on a 0-7 scale on 14 Sept. 1940, the average being taken as the true value. Table 10 gives the Striga estimates obtained in 1939 and the increase or decrease over the 1939 values shown in the 1940 estimates.

An analysis of variance and co-variance of the results showed that the effect of the light watering was highly significant, and that highly infected subplots in the 1939 estimate were highly infected in the

1940 estimate (correlation coefficient + 0.924). The treatment, therefore, has not altered the relationship of *Striga* intensity between subplots but, in the light-watering series, the increase in intensity has been greater than in the heavy-watering series.

At the conclusion of this experiment it was decided to ascertain if this differential watering effect persisted if sorghum was sown out of season.

In the first experiment sixty petrol tins were filled with soil mixed with 0.4 g. *Striga* seeds per tin. All tins were sown with sorghum on 19 Nov. 1940: thirty tins were given a light watering and thirty tins received double the watering of the light-watered tins. The number of *Striga* plants that appeared was counted and the experiment ended on 4 Feb. 1941. The total *Striga* appearances per treatment were: heavy-watering series 105 plants, light-watering series 167 plants. The difference is not significant.

In the second experiment, the sorghum was sown on 10 June 1941 and the experiment ended on 27 Aug. 1941. The results obtained were: heavy-watering series 763 *Striga* plants, light-watering series 1495 plants. The difference is significant. In this experiment the sorghum plants were removed from all tins, the top soil broken up and the tins again sown with sorghum seed, but all tins were given an *equal* watering. The total *Striga* appearances after 2 months' growth were 242 plants in the previously *heavily* watered tins and 408 *Striga* plants in the previously *lightly* watered tins. The difference is highly significant, indicating that whatever was the effect on *Striga* attack produced by the lighter watering, it persisted when a further crop of sorghum was grown and the two series received equal waterings. The *Striga* plants in the original experiment were not allowed to seed, and it was expected that less *Striga* would have appeared in the tins that had previously received a light watering since the number of germinable *Striga* seeds left after the original experiment is likely to be less there than in the heavily watered series. It seems clear that in the original experiment only a small proportion of available germinable *Striga* seed was germinated in the experiment. Since the differential effect persisted, even when sorghum plants were given equal watering, it appears that the treatments have induced some soil changes which rendered the sorghum plants in the light-watered series more susceptible to *Striga* attack, or which incapacitated some of the *Striga* seeds in the heavily watered series.

A third experiment was carried out when the sorghum seed was sown on 3 Nov. 1941. No significant difference in total *Striga* appearances was found.

It seems clear, therefore, that whatever is the change induced by differential watering, it is only significant when the sorghum is sown at its normal sowing time, viz. during the early part of the rainy season (June–Oct.).

DISCUSSION

Striga hermonthica has not as far as is known spread to the vast sorghum-growing areas of America, but is confined to countries which include those from which sorghum is believed to have originated.

Though this paper describes *Striga* under irrigation, this parasite occurs in the Sudan equally severely on rain-grown sorghum. Saunders (1933) noted that *S. asiatica* is common on impoverished land. Observations in the Sudan on rain land have shown that as the land becomes progressively infertile owing to continuous cropping with sorghum, the infestation with *S. hermonthica* becomes increasingly severe until a stage is reached when it is no longer profitable to grow sorghum. In rain-grown areas in the Sudan, where shifting cultivation is practised, the appearance of *Striga* is often taken as a sign that the cultivation should be removed to a more fertile area. As was previously stated, under irrigation in the Gezira cotton area, a close rotation was found to induce a severe *Striga* attack. When two successive fallows were introduced into the rotation the increased fertility which resulted from this change caused the *Striga* attack to become less and, at present, *Striga* on sorghum in the irrigated Gezira is considered a minor pest. Following improves the physical condition of Gezira soil and its ability to supply nitrogen to the crop. It has been shown by Dr F. Crowther, Chief Plant Physiologist on the Research Farm, that the application of sulphate of ammonia to the irrigated sorghum crop decreases the *Striga* attack. It thus appears that sorghum is more resistant to *Striga* attack when conditions favouring growth of the crop are improved either by fallowing or by addition of a nitrogenous fertilizer. The exact mechanism involved is obscure. It has been shown above that the number of *Striga* plants attached to the root of a sorghum plant is proportional to the root's development when the *Striga* seed is evenly distributed through the soil. This would suggest that, unless the innate resistance of a plant is increased by the nitrogen, the root system of the sorghum is changed. We have as yet no knowledge that there is not a change, induced by the nitrogen, in the activating power of the excretions of the sorghum root. It is possible that the increased *Striga* attack under light watering obtained in the differential watering experiment may be due to some soil change involving the activities of the soil microflora. Investigations are now proceeding on these aspects of the *Striga* problem.

The varieties of plants whose root excretions have been found to germinate *S. hermonthica* seed are noteworthy. This list can represent only a small proportion of such plants. An extremely small percentage germination of *Striga* seeds can be obtained by using water alone, but the greater majority require stimulation. The nature of the stimulating principle is not known, but its occurrence is probably wide-

spread in the plant kingdom. The germination of the *Striga* seed by a non-parasitized leguminous crop suggests that besides acting as a soil improver, the leguminous plants may cause the death of *Striga* seeds in the soil and assist in ridding the land of this parasite. The effect of a leguminous crop on *Striga*-infested land is now being investigated.

I wish to acknowledge the assistance received from our Senior Technical Assistant, Zein Eff, Abdel Nabi and other members of the staff in the collection of the data, and also the help received from Mr H. E. King, Chief Plant Breeder, in the statistical analysis of the differential watering experiment.

REFERENCES

- DOUGHTY, L. R. (1942). A note on *Striga* investigations at Amani. *E. Afr. agric. J.* **8**, 33.
 JOSEPH, A. F. (1925). Alkali investigations in the Sudan. *J. agric. Sci.* **15**, 407.
 ROSE, M. F. & LOCHRIE, J. V. (1941). Witchweed: *Striga asiatica* (L.) D. Kuntze (= *S. lutea* Lour.). Report on laboratory germination of seed. *Emp. Cotton Growing Corp. Progr. Report*, 1939-40, p. 32.
 SAUNDERS, A. R. (1933). Studies in phanerogamic parasitism with particular reference to *Striga lutea* Lour. *Sci. Bull. Dep. Agric. Union of S. Afr.* no. 128.
 STEPHENS, E. L. (1912). The structure and development of the haustorium of *Striga lutea*. *Ann. Bot., Lond.*, **26**, 1067-76.

(Received 29 January 1945)

Addendum. Since this paper was accepted by the Editors, Brown & Edwards have published a letter in *Nature* (Brown, R. & Edwards, M., 'Effects of thiourea and allylthiourea on the germination of seed of *Striga lutea*', *Nature*, 1945, 14 April, p. 455),

stating that in the absence of the host stimulant a small percentage of germination of seeds of *Striga lutea* is obtained in culture vessels when seeds are moistened with solutions of thiourea and a slightly higher percentage with allylthiourea.

The influence of date of lifting and method of storing on loss of onion bulbs harvested in 1943

BY E. R. WALLACE, *The Agricultural Institute, Kirton, Boston, Lincs*
 AND C. J. HICKMAN,* *Botany Department, The University, Birmingham*

(With 1 Text-figure)

Date of lifting experiments with four varieties of onion at two centres are examined in relation to the incidence of losses in store, and at one centre the effects of indoor and outdoor storage are compared. Losses due to *Botrytis*, eelworm infection and sprouting are treated separately, and the practical significance of the results is discussed.

INTRODUCTION

One of the results of the huge expansion of bulb-onion growing in this country during the war has been to emphasize the problems involved in the production of the crop, and amongst these, the occurrence of several diseases for which control measures are lacking or imperfect. One of the most serious of the diseases that attack onions, and, in fact, the most important cause of loss in store, is neck rot. Munn (1917) attributed the disease to *Botrytis Allii*, but Walker (1926) described in addition two other, though less common, American types of neck rot, differing slightly from that due to *B. Allii*, and caused

by *B. byssoidea* and *B. squamosa* respectively. In this country, *B. Allii* is by far the most common neck rot organism, but a fungus agreeing with *B. byssoidea* has been isolated recently from stored bulbs (Croxall & Pickford, 1944). Although *B. squamosa* has frequently been identified on onion foliage in England (Hickman & Ashworth, 1943), it has not yet been encountered on onion bulbs.

Walker showed that under comparable conditions onions with the neck tissues well dried off were less susceptible to natural infection than those with neck tissues that were still succulent. He suggested that cool, wet weather, by providing favourable conditions for the development of the parasite and delaying the ripening of the bulb, increased the amount of neck rot, while hot, dry weather hampered *Botrytis* development and promoted rapid and thorough desic-

* This joint investigation was carried out during the service of the second author as a Scientific Officer of the Agricultural Research Council.

cation of the neck tissues. That the incidence of neck rot was not solely related to degree of maturity was shown by an experiment in which immature bulbs, harvested during good curing weather, kept better than mature bulbs harvested later but exposed to conditions more favourable to infection. A number of horticulturists in this country support the general statement that early harvested onions will keep better, but such a statement lacks value in the absence of precise information concerning increases of crop weight lifted as the season advances, as well as of comparative losses in store.

In 1943 experiments on the lifting of onions at different dates were laid down at Kirton in Lincolnshire and Perdiswell in Worcestershire. The varieties used were the same and the seed was from the same stocks; the lay-out also followed the same general plan at both places. Further advantage for comparing the two sets of results is afforded by the fact that the produce from these two centres was kept well into 1944, thus yielding results over an extended storage period. Although the experiments were designed primarily to investigate the effect of the treatments on neck rot, two other factors causing losses in store had to be taken into account, namely, rotting caused by eelworm, *Anguillulina dipsaci*, and premature sprouting.

EXPERIMENTAL

At both centres, Kirton and Perdiswell, seed was sown in the spring and the crop was harvested in three successive stages, at intervals of 3 weeks. After the bulbs from each lifting had dried off, they were placed in store. At Kirton all the bulbs were stored indoors; at Perdiswell a comparison was made between indoor and outdoor storage. Information on the causes and progress of loss during the storage period was obtained by counting and removing all but sound bulbs at intervals throughout the winter and early spring, the weight of healthy bulbs surviving being recorded at each examination.

The experiment was carried out with four varieties, Ailsa Craig, Bedfordshire Champion, Unwin's Reliance and Up-to-Date (referred to below as A, B, R and U). There were nine replications of each treatment for each variety, in randomized blocks, each single variety plot consisting of three rows, 6 ft. in length. At Kirton the plot of land chosen for the experiment had not borne onions for at least 13 years. In 1942 the crop was wheat. No fertilizer was applied for the onion crop, seed of which was sown on 23 Mar. in drills 10 in. apart. At Perdiswell the land had borne runner beans in 1942. A basal dressing of meat and bone meal (at the rate of 8 cwt./acre), crushed hoof (4 cwt./acre) and muriate of potash (2 cwt./acre) was applied on 25 Feb., and seed was sown on 3 Mar. in drills 14 in. apart.

Liftings were made at Kirton on 26 July, 16 Aug. and 6 Sept. The bulbs were placed in boxes and left

outside to dry. The boxes were not stacked. Those from the first lifting were taken into store in an unheated shed on 17 Aug., from the second lifting on 7 Sept., and from the third on 27 Sept. At Perdiswell liftings were made on 6 Aug., 27 Aug. and 16 Sept. At the time of the first lifting the leaves were green but falling over. By 27 Aug. the leaves were prostrate and beginning to dry off, while on 16 Sept., with the exception of Reliance, which still showed a few green leaves, the tops were dead and withered. The soil was now damp and some re-rooting had occurred in all four varieties. After loosening the bulbs by forking underneath, they were pulled up by hand, carried to another piece of ground, and laid out to dry. The bulbs of the three liftings were picked up and placed in store, in wide-meshed string bags, on 27 Aug., 15 Sept. and 11 Oct. respectively. The indoor store was an unheated wooden shed. The outdoor store consisted of a V section wire-netting enclosure supported by stakes, lined at the bottom with brushwood and roughly protected above by corrugated iron sheeting.

The Kirton crop was examined on five occasions, beginning on 5 Oct., 1 Nov., 6 Dec., 24 Jan. and 27 Mar. Four examinations were made at Perdiswell beginning on the following dates: 1 Nov., 6 Dec., 17 Jan. and 29 Feb. The rejected bulbs were sorted into three main categories, (1) attacked by *Botrytis*, (2) attacked by eelworm, and (3) prematurely sprouted bulbs. In addition, some bulbs were infected by *Penicillium* spp. and bacterial soft rots, but the numbers of these were so small that they are not considered further.

To obtain figures in each of the above categories that would be representative of their independent importance, the total percentage losses by number were adjusted by means of the formula $\frac{100X}{(H+X)}$, where H is the number of sound bulbs and X the number respectively attacked by *Botrytis*, or by eelworm, or of those sprouted prematurely. Data were also obtained on the final surviving weight and on the combined percentage loss of healthy weight. The results were analysed statistically.

RESULTS

Crop weight

The increase in weights lifted as the season advanced, and the course of survival in store of the onions from each of the liftings may be followed in Fig. 1. It will be seen that the increase of crop weight in the respective 6-week intervals covered by these liftings was much greater at Kirton than at Perdiswell. Although at both centres the later the lifting the more rapid was the subsequent loss, the final results at the two centres were in striking contrast. Whereas at Kirton the final weight surviving was lowest from

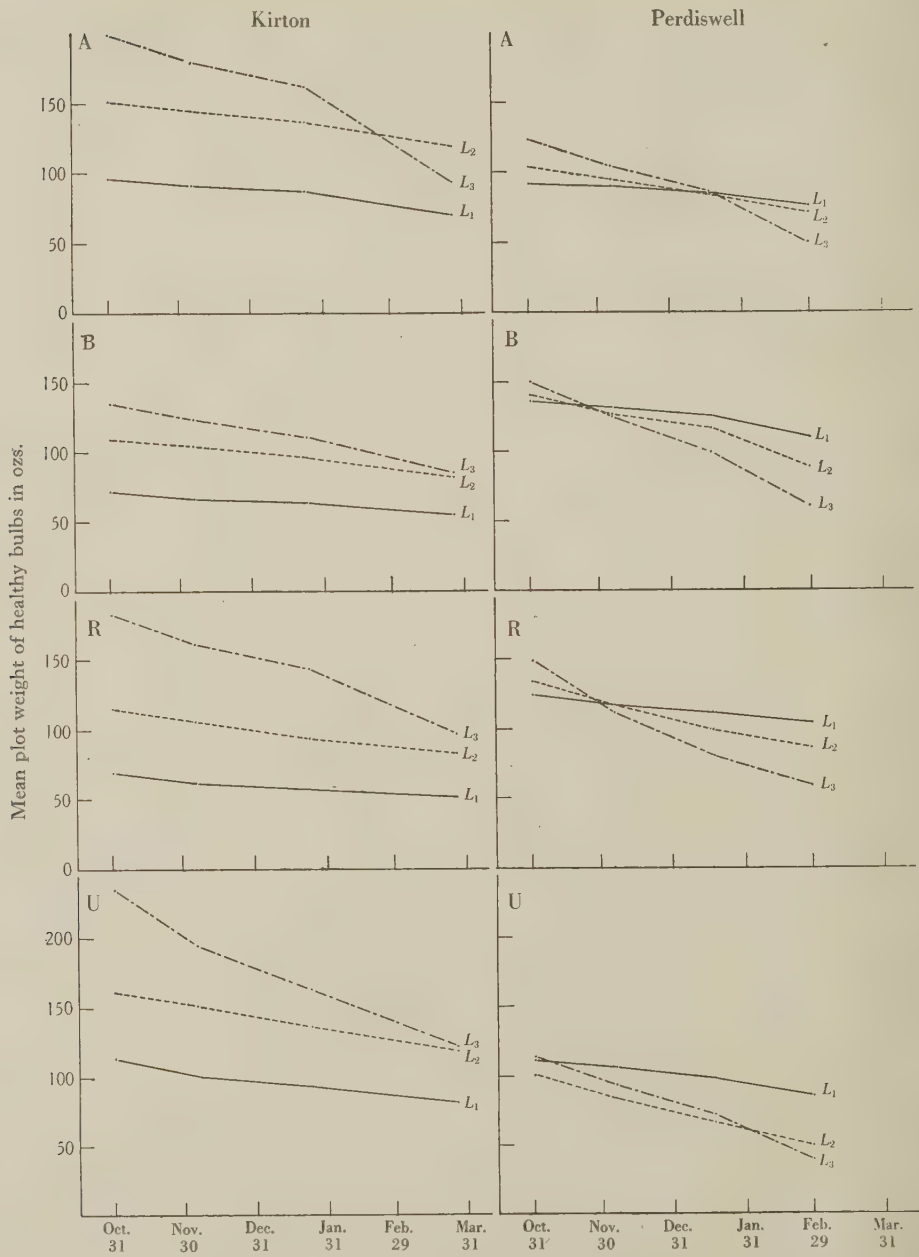


Fig. 1. Illustrating the influence of date of lifting on crop weight and the course of survival of the crop from each lifting at Kirton and at Perdiswell.

A, Ailsa Craig. B, Bedfordshire Champion. R, Unwin's Reliance. U, Up-to-Date.

the first lifting in every variety, at Perdiswell the original order of crop weights, $L_3 > L_2 > L_1$, became completely reversed. At Kirton the losses from the first and second liftings ran parallel in all four varieties, but the third lifting lost more heavily, causing the final weights therefrom to approach those from the second liftings in R and U and fall below them in A and B.

By retaining the letters for liftings (see Table 1) and varieties, and adopting a convention for numerical results, further contrasts may now be indicated:

Unanalysed results:

A > B A is numerically greater than B.

Analysed results:

A ~ B A is numerically greater than B, not significantly so,

A * B A is significantly greater than B (5% point),

A ** B A is significantly greater than B (1% point).

In crop weight, as lifted, whereas at Kirton $L_3 > L_2 > L_1$ and $U > A > R > B$, at Perdiswell $L_3 > L_2 > L_1$ but $B > R > U > A$. In final healthy weight the Kirton experiment gave $L_2 \sim L_3$ * L_1 and $(U \sim A)$ * $(R \sim B)$ also A * * B; Perdiswell L_1 * * L_2 * * L_3 , and $(B \sim R)$ * * $(A \sim U)$. The total loss as a percentage of the healthy weight shows at Kirton L_3 * * $(L_1 \sim L_2)$, $R \sim U \sim A \sim B$, R * * $(A \sim B)$ and U * * B, but at Perdiswell L_3 * * L_2 * * L_1 and $(U \sim B)$ * * A, and R * A, and $B \sim R$.

Sources of loss

Of the three main sources of loss, neck rot was of chief importance, followed in order by premature sprouting and eelworm.

(a) *Neck rot*. At Kirton in the total percentage loss by number $L_3 \sim L_1 \sim L_2$ and L_3 * * L_2 also R * $U \sim A \sim B$, R * * B and U * * B. At Perdiswell L_3 * * L_2 * * L_1 and $U \sim B \sim R$ * * A. At this centre the low total loss in A reflects the relatively small loss in the produce from the second lifting, in which this variety differs significantly from the other three (Table 2).

(b) *Premature sprouting*. At both centres the losses under this head were relatively small and not very different in the onions from first and second liftings. But the third lifting showed a large increase: Kirton L_3 * * $L_1 \sim L_2$, Perdiswell L_3 * * L_2 * * L_1 . The low figures in U at Kirton are a significant exception (Table 2).

(c) *Eelworm*. At Kirton $L_3 \sim L_1 \sim L_2$, but at Perdiswell L_3 * * L_2 * * L_1 . The variety Up-to-Date showed heavy infection, significantly greatest at both centres; Kirton U * * $B \sim R \sim A$, Perdiswell U * * B * A ~ R. This point is discussed more fully later. At Perdiswell the general trend between liftings is a reflexion of significant differences in the three varieties A, B, U considered independently; the lower figures in R do not reach the 5% level for significance (Table 2).

Indoor and outdoor storage

In this set of comparisons at Perdiswell there was no significant difference in the amount of loss from *Botrytis* developing indoors and outdoors, but the losses due to sprouting and eelworm were significantly reduced by outdoor storage as will be seen from Table 3.

The difference in loss from these two sources, as between indoor and outdoor store, was negligible in the produce from first and second liftings, but from the third lifting, with much higher losses, outdoor storage displayed a significant (**) advantage in both cases (Table 4).

DISCUSSION

Eelworm attack was an unexpected but interesting complication, as the figures from the three liftings confirm the observation that this pest continues to attack fresh bulbs up to the end of the growing season. The marked difference between U and the other varieties is attributable at both centres to infestation of the seed by larval eelworms. These were found in samples of the seed left over from the Kirton sowing (Goodey, 1943; Wallace & Wood, 1943) and the Perdiswell seed was of the same stock. At Kirton only four plants were found affected in the field, but at Perdiswell the attack was more obvious, and it is possible that the relatively greater losses in the varieties A, B and R at this centre were due to spread in the field.

The differences in sprouting in store from the three liftings were significantly of the same kind under three different sets of storage conditions (indoor at Kirton, indoor and outdoor at Perdiswell), earlier sprouting following later lifting. Although no explanation can be offered, the contrast in the behaviour of the varieties at the two centres is noteworthy.

No attempt has been made to examine the reasons underlying the differences in the losses due to *Botrytis* between liftings, if only because it is unwise to generalize from the results of one season's work. The results obtained do, however, provide evidence in support of the view that the proportion of loss is increased by later lifting. At the same time, early lifting has provided no solution to the economic problem of *Botrytis* loss, since losses from the first lifting were quite high. The Perdiswell figures show that fears need not be entertained about storing onions in a suitable manner in the open, because outdoor storage did not lead to any increase in losses. In conclusion, the results illustrate the danger of applying local experimental results directly to general practice. In spite of the same general trend in storage loss at both centres, the comparisons between initial yields, which latter determine the saleable residue at any given time, were very different as between Kirton and Perdiswell.

TABLE I. *Results to end of storage period*

	Liftings				Varieties									Sig. inter- action
	L_1	L_2	L_3	Mean	S.E.	Sig. diffs.	A	B	R	U	Mean	S.E.	Sig. diffs.	
Surviving healthy weight at 27 Mar. oz./plot mean	64.61	100.50	99.00	88.00	4.74	Kirton	93.74	72.00	77.22	107.19	88.00	5.48	15.41* 20.44**	—
% loss of healthy weight first to last weighing	35.50	33.29	54.51	41.10	1.74		38.80	34.87	46.93	43.80	41.10	2.01	5.65* 7.50**	$L \times V$ (Table 2)
Total % loss by number due to <i>Botrytis</i> infections	28.64	23.29	33.94	28.63	2.39		24.21	21.06	38.74	30.53	28.63	2.46	6.92* 9.18**	—
Total % loss by number due to sprouting	4.20	3.18	16.13	7.83	0.85		12.58	7.18	7.66	3.91	7.83	0.99	2.77* 3.68**	$L \times V$ (Table 2)
Total % loss by number due to eelworm infections	(4.88)	(4.11)	(8.77)	(5.92)	—		0.91	2.30	1.46	19.00	5.92	1.99	5.61* 7.44**	—
Surviving healthy weight at 29 Feb. oz./plot mean	93.40	73.97	51.47	72.94	2.72	Perdiswell	65.01	85.87	83.01	57.88	72.94	3.12	8.69* 11.47**	—
% loss of healthy weight first to last weighing	27.89	48.99	68.34	48.41	1.44		41.90	50.34	46.59	54.79	48.41	1.66	4.65* 6.14**	$L \times V$ (Table 2)
Total % loss by number due to <i>Botrytis</i> infections	19.20	38.03	52.15	36.45	1.69		27.33	39.25	39.01	40.25	36.45	1.95	5.44* 7.17**	$L \times V$ (Table 2)
Total % loss by number due to sprouting	4.04	7.56	40.06	17.22	1.05		18.59	15.81	13.24	21.24	17.22	1.22	3.37* 4.45**	$L \times S$ (Table 4)
Total % loss by number due to eelworm infections	2.10	6.59	14.2	7.64	0.90		5.06	8.21	2.50	14.75	7.63	1.04	2.89* 3.82**	$L \times V$ (Table 2)

*—minimum sig. diff. (5% point)

**—minimum sig. diff. (1% point)

brackets—results not sig. by z test

TABLE 2. *Interactions—liftings × varieties*

Interactions		L_1	L_2	L_3	Mean	S.E.	Sig. diffs.
Kirton							
% loss of healthy wt./plot mean	A	31.38	26.08	58.94	41.10	3.48	9.79*
	B	31.54	28.63	44.43			12.99**
	R	41.84	41.17	57.78			
	U	37.22	37.29	56.89			
% loss due to sprouting/plot mean (on number)	A	6.93	4.09	26.73	7.83	1.71	4.81*
	B	5.93	3.57	12.04			6.37**
	R	1.82	3.37	17.80			
	U	2.11	1.67	7.94			
Perdiswell							
% loss of healthy wt./plot mean	A	24.31	36.58	64.70	48.41	2.89	8.04*
	B	32.75	51.23	67.03			10.01**
	R	24.35	45.76	69.70			
	U	30.05	62.40	71.93			
% loss due to <i>Botrytis</i> /plot mean (on number)	A	16.00	21.63	44.36	36.45	3.38	9.42*
	B	26.11	41.35	50.29			12.43**
	R	18.21	40.32	58.51			
	U	16.49	48.84	55.43			
% loss due to eelworm/plot mean (on number)	A	1.62	2.36	11.20	7.63	1.80	5.00*
	B	1.30	6.80	16.53			6.60**
	R	0.48	1.29	5.73			
	U	5.01	15.91	23.33			

TABLE 3. *Comparisons from indoor and outdoor storage*

	Indoor	Outdoor	Mean	S.E.	Sig. diffs.	Sig. interaction
% loss by number due to sprouting	18.71	15.74	17.22	0.86	2.40*	$L \times S$
					3.17†	
% loss by number due to eelworm	9.04	6.22	7.63	0.73	2.11*	$L \times S$
					2.78†	

TABLE 4. *Interactions—liftings × storage*

Interactions		L_1	L_2	L_3	Mean	S.E.	Sig. diffs.
% loss due to sprouting/plot mean (on number)	Indoor	2.64	7.76	45.71	17.22	1.49	4.16*
	Outdoor	5.45	7.35	34.41			5.48**
% loss due to eelworm/plot mean (on number)	Indoor	1.82	6.62	18.69	7.63	1.27	3.55*
	Outdoor	2.39	6.56	9.70			4.68**

The authors wish to express their gratitude to all those who assisted in the experiments, and, in particular, to J. C. Wallace, Principal, Kirton Agricultural Institute, and L. F. Clift, Horticultural

Superintendent, Worcestershire County Council, who provided the opportunity to carry out this investigation.

REFERENCES

- CROXALL, H. E. & PICKFORD, P. T. H. (1944). Manurial experiments on vegetable crops. VI. Effects of farmyard manure and other manurial treatments on yield and storage rots of onions. *Rep. agric. hort. Res. Sta., Bristol*, for 1943, pp. 57–61.
- GOODEY, T. (1943). *Anguillulina dipsaci* in the inflorescence of onions and in samples of onion seed. *J. Helminth.* **21**, 22–30.
- HICKMAN, C. J. & ASHWORTH, DOROTHY (1943). The occurrence of *Botrytis* spp. on onion with special reference to *B. squamosa*. *Trans. Brit. mycol. Soc.* **26**, 153–7.
- MUNN, M. T. (1917). Neck-rot disease of onions. *Bull. N.Y. St. agric. Exp. Sta.* no. 437, pp. 363–455.
- WALKER, J. C. (1926). *Botrytis* neck-rots of onions. *J. agric. Res.* **33**, 893–928.
- WALLACE, E. R. & WOOD, J. (1943). Stem eelworm in onion bulbs, probably seed-borne in origin. *J. Helminth.* **21**, 33–6.

(Received 22 January 1945)

Black scurf and stem canker of potato (*Corticium Solani* Bourd. & Galz.)

Further field studies on the use of clean and contaminated seed potatoes and on the contamination of crop tubers

By T. SMALL, PH.D., A.R.C.S., *Agricultural Advisory Department, Manchester University*

Black scurf and stem canker of the potato were investigated in field trials in contaminated soil at Warburton, Cheshire, to determine the effect of planting clean and contaminated seed (vars. Arran Banner and Majestic) on crop yield and on the contamination of the crop tubers.

Black-scurf contamination was prevalent on crops grown from clean seed and was not significantly different from that occurring on plots planted with contaminated seed. It was severe on early-dug tubers, but was more severe on late-dug tubers. Produce grown with and without stable manure was heavily contaminated.

Contaminated seed caused a check to tuber formation and an appreciable increase in stem canker and in the number of primary shoots killed. Nevertheless, the yields from the manured, contaminated seed plots were satisfactory and were not lower than those from the clean-seed plots. There was no relation between the yield and the amount of black scurf on the produce.

The results of a trial made in a field which had been in grass for at least 43 years, suggested that the soil contained little, if any, *Corticium Solani*. Heavy contamination occurred on tubers grown under relatively dry soil conditions.

In all the trials missses and wilted shoots caused by *Corticium* were rare and there was no premature yellowing or death of the haulms on the clean or contaminated seed plots.

The evidence obtained in the four seasons from 1941 to 1944 indicates that in this country satisfactory yields of early maincrop and maincrop varieties may be obtained despite the prevalence of *C. Solani* in the soil and on the seed, provided the soil and cultural conditions are reasonably good.

EXPERIMENTAL

The studies described in an earlier publication (Small, 1943) were continued in 1943 and 1944 at Warburton, Cheshire. The plots used in 1943 had been in potatoes 1940, carrots 1941, and oats 1942; those used in 1944 had been in grass 1941, potatoes 1942, and silage mixtures in 1943. The pH of the soil in April was 5.12 in 1943 and 5.71 in 1944. After the usual ploughing, cultivating and ridging, stable manure (where used) was applied in the drills (10 tons/acre), the ridges were split back and the sets planted (3 May 1943, and 27 Apr. 1944) 4 in. deep along the ridges. Unless otherwise stated 2 oz. sets having shoots $\frac{1}{2}$ –1 in. long were planted; twice-grown seed was used in 1943 and once-grown in 1944. The rows were 27 in. apart, each contained twenty-five sets 12 in. apart, and each received 1 lb. of a standard potato fertilizer before the final ridging in June. The 1943 season was early and favourable for growth; 1944 was not early and severe frosts in May killed all the shoots above ground. In each year the frequent incidence of the spore stage of *Corticium* on all the plots indicated that the fungus was prevalent in the soil.

Harvesting, tuber examination and classification were carried out as described earlier (Small, 1943). In the tables the total yields are recorded and the figures in the 'black scurf' columns include only heavily contaminated tubers, and these are expressed as percentages of the total number of potatoes

examined. The varieties Arran Banner and Majestic were used; early varieties were not included because in this area little black scurf occurs on own-grown seed.

SOIL MOISTURE AND TEMPERATURE

The method of soil sampling described earlier (Small, 1943) was followed. Table 1 summarizes the maximum and minimum soil-moisture values (expressed as percentages of the wet samples) obtained at each sampling of the stable-manure plots. In 1944 plots without stable manure were tested separately, and the moisture values were usually 2–3 % lower than on the stable-manure plots. The soil temperatures (4 in. deep) are taken from the weekly mean figures recorded in the Warburton Registers of the Agricultural Meteorological Scheme.

The soil moisture was higher in 1944. In 1944 the weekly mean temperatures were slightly higher (1–2°F.) in May, July and August, slightly lower in June, and lower (2–6°F.) in September than in the corresponding months of 1943.

EFFECT OF PLANTING CLEAN AND CONTAMINATED SEED ON BLACK SCURF AND CROP YIELD

In 1943 the trial consisted of eight two-row plots (four with stable manure and four without) of Arran Banner; in each plot one row was planted with clean and the other with heavily contaminated seed. The

crop was lifted 27 Sept. and the tubers, 1629, were examined. The test was repeated in 1944 and the tubers, 3384, were lifted and examined 2-4 Oct. In each year the clean-seed rows were the more vigorous early in the season, but in July the difference had disappeared. No wilted shoots occurred; no misses were noted in 1944 and only two in 1943. The results are given in Table 2.

heavier in 1944, possibly due to the higher soil moisture. On both varieties and in each season the yields from clean seed were no greater than those from contaminated seed, and the latter gave a satisfactory yield on the dunged plots even in the unfavourable 1944 season. There was no relation between the yield and the amount of black scurf on the produce.

TABLE 1. *Soil moisture and temperature in trial plots*

No. weeks after planting	Soil moisture (%)				Month	Soil temperature (°F.)			
	1943		1944			1943		1944	
	Min.	Max.	Min.	Max.		Min.	Max.	Min.	Max.
1	19.4	23.3	22.6	23.1	May	44.7	57.9	46.0	60.3
3	18.4	21.9	20.8	22.7	June	54.0	60.7	51.9	60.3
5	16.0	19.5	20.3	21.7	July	56.2	64.0	56.7	63.5
8	12.6	14.7	16.2	19.2	Aug.	55.4	65.2	55.5	64.0
13	5.0	8.8	12.4	13.2	Sept.	48.0	60.7	43.0	57.9
16	10.7	14.8	16.4	18.6					
21	17.4	19.6	23.6	25.7					

TABLE 2. *Effect of planting clean and contaminated seed on black scurf and crop yield*

Row no.	Stable-manure plots								No stable-manure plots							
	Clean seed				Contaminated seed				Clean seed				Contaminated seed			
	Black scurf		Yield lb.		Black scurf		Yield lb.		Black scurf		Yield lb.		Black scurf		Yield lb.	
	1943	1944	1943	1944	1943	1944	1943	1944	1943	1944	1943	1944	1943	1944	1943	1944
1	28	60	47	47	52	49	45	45	28	65	41	34	49	68	34	36
2	33	73	57	39	54	64	46	51	36	68	33	30	53	76	37	31
3	65	73	56	38	68	69	56	41	56	72	39	28	54	77	45	29
4	74	73	49	43	68	71	59	44	74	80	32	30	65	78	38	29
5	—	73	—	37	—	72	—	38	—	81	—	24	—	78	—	28
6	—	82	—	36	—	74	—	44	—	84	—	25	—	86	—	25
Total yield			209	240*			206	263			145	171			154	178

* 10.4 tons/acre.

EFFECT OF PLANTING CLEAN AND CONTAMINATED SEED ON BLACK SCURF AND CROP YIELD; VARIETY TRIAL

On 3 May 1943 eight rows of clean and eight of heavily contaminated seed of each of the varieties Arran Banner (a vigorous variety) and Majestic (a less vigorous variety) were planted in plots manured with stable manure. On 2 June there was more growth on the clean-seed plots, but on 8 July the difference was scarcely discernible; no wilted shoots and only one miss were recorded. The tubers, 3603, were lifted and examined 28-30 Sept. (Table 3).

The results given in Tables 2 and 3 show that the amount of black scurf on the produce was considerable on the clean-seed plots and was not significantly different from that occurring on the contaminated seed plots. The contamination was heavy on the areas with and without stable manure, and was far

In 1943 far fewer tubers, excluding chats, were produced on plants raised from contaminated seed, but in 1944 the numbers from the clean and contaminated plots were almost equal; thus the suggestion made earlier (Small, 1943), that black scurf may act as a pruning agent causing fewer but larger tubers to be produced, was supported in 1943 but not in 1944. It is possible that the results in 1944 were affected by the May frosts.

EFFECT OF PLANTING CLEAN AND CONTAMINATED SEED; ATTACK ON PRIMARY AND GROWING SHOOTS AND TUBER FORMATION

In 1943 four rows of clean and four of heavily contaminated seed of each of the varieties Arran Banner and Majestic were planted on 3 May; these rows were contiguous with those of the trial summarized

in Table 3 and had been manured with dung. On 21-22 June, 7 weeks after planting, the plants were lifted and examined for stem canker and tuber formation; the results are given in Table 4.

In 1944 four two-row plots (two with stable manure and two without) of Arran Banner were planted alongside the trial summarized in Table 2; in each plot one row was planted with twenty-five clean seed and the other with twenty-five contaminated. The May frosts killed many primary shoots and caused numerous side shoots to develop; it was

formation were caused mainly by the fungus on the seed and not by that in the soil. The results further suggest that the plants raised from contaminated seed in the trials summarized in Tables 2 and 3 also suffered in the early stages; recovery from these attacks (as shown by the yields) occurred on the plots with and without stable manure and on both the varieties Arran Banner and Majestic. Recovery from early attacks may be less complete in early varieties owing to the shorter growing period, but this has not been tested because in this area, although

TABLE 3. *Effect of planting clean and contaminated seed on black scurf and crop yield; variety trial*

Row no.	Black scurf				Yield (lb.)			
	Clean seed*		Contaminated seed		Clean seed		Contaminated seed	
	Arran Banner	Majestic	Arran Banner	Majestic	Arran Banner	Majestic	Arran Banner	Majestic
1	33	18	46	42	48	57	44	51
2	43	24	52	45	54	49	49	50
3	45	34	52	47	56	48	52	49
4	51	34	54	48	58	49	53	53
5	53	45	55	51	45	50	52	47
6	58	51	58	55	47	59	58	42
7	69	63	61	55	47	59	53	57
8	85	69	62	71	57	48	48	53
Total yield					412	419	409	402†

* 3 oz. size seed of Majestic were planted.

† 13.0 tons/acre.

TABLE 4. *Effect of planting clean and contaminated seed; attack on primary and growing shoots and tuber formation*

Variety	No. primary shoots killed	Growing shoots		No. tubers
		No.	No. attacked	
Arran Banner*	6 (6)†	222 (100)	22 + 6† (18)	642 (88)
Majestic*	3 (3)	342 (100)	31 + 26 (28)	841 (96)
Arran Banner§	82 (58)	265 (100)	34 + 22 (38)	255 (66)
Majestic§	40 (28)	326 (99)	48 + 45 (51)	448 (76)

* Clean seed.

† 100 clean and 100 contaminated seed of each variety were planted; in each column the figures in brackets show the number of plants contributing to the result given.

‡ The first figure denotes a small lesion ($\frac{1}{4}$ – $\frac{1}{2}$ in. long) the second a larger lesion (usually more than $\frac{3}{4}$ in. long).

§ Contaminated seed.

decided, therefore, to limit the examination, made 9 weeks after planting, to a count of the young tubers (see Table 5).

The results given in Table 4 show that only nine primary shoots were killed on the clean-seed plots whereas 122, distributed over eighty-six plants, were killed on the contaminated seed plots; stem canker was more prevalent on the latter plots. Tables 4 and 5 show that the number of young tubers (minimum size $\frac{1}{4}$ in. diam.) was far higher on the clean-seed plots. The results indicate that, despite the prevalence of *Corticium* in the soil of all the plots, the attack on the primary shoots and the check to tuber

the soil is heavily contaminated, little black scurf occurs on own-grown seed which is usually lifted in July or early August. Early varieties lifted later in the season, as in Scotland or Northern Ireland, have not been examined for black scurf.

TABLE 5. *Effect of planting clean and contaminated seed on tuber formation: Arran Banner*

Seed; plot treatment	No. tubers
Clean seed; stable manure	563
Contaminated seed; stable manure	230
Clean seed; no stable manure	472
Contaminated seed; no stable manure	252

RELATION BETWEEN DATE OF LIFTING,
BLACK SCURF, AND YIELD

On 3 May 1943 fifteen rows, all manured with dung, were planted with clean, 3 oz. size seed of Arran Banner. At 3-week intervals beginning 30 Aug. five rows were dug, and the tubers, 2077, weighed and examined (see Table 6).

TABLE 6. *Showing relation between date of lifting, black scurf, and crop yield (1943)*

Row no.	Date lifted and black scurf			Date lifted and yield (lb.)		
	30 Aug.	20 Sept.	11 Oct.	30 Aug.	20 Sept.	11 Oct.
1	17	25	31	47	62	64
2	18	42	50	44	55	54
3	21	48	69	58	55	56
4	36	59	77	55	66	59
5	56	71	82	46	48	59
Total yield	—	—	—	250*	286†	292

* 12.9 tons/acre.

† 14.8 tons/acre.

Although black scurf occurred least on the earliest dug and most on the latest dug, the former were rather heavily contaminated despite the use of clean seed. Good crop yields were obtained and a considerable increase occurred from the first to the third week in September. No relation was apparent between the yield and the amount of tuber contamination. The results suggest that attempts to obtain clean seed of early maincrop and maincrop varieties by early lifting are likely to prove disappointing and to be attended by serious loss in yield.

EFFECT OF PLANTING CLEAN AND CONTAMINATED
SEED AFTER OLD GRASS

This test was made in 1943 in a field which had been in old turf for at least 43 years. On 3 May five rows were planted with clean seed and five with contaminated; the plots, all without dung, were lifted 29 Sept. and the tubers (1709) were examined for black scurf. The percentage of heavily contaminated tubers on the clean and contaminated seed plots respectively was 7 and 65; the latter figure shows that the soil conditions were not unfavourable for the fungus. No sclerotia were visible on the clean seed at planting time, but hyphae may have been present and may have caused some of the contamination on the clean-seed plots. The result suggests that the soil was almost, if not entirely, free from *Corticium Solani*; the effect of planting contaminated seed in such soil is clearly shown.

OCCURRENCE OF BLACK SCURF IN SOILS
WITH A LOW MOISTURE CONTENT

In 1944 110 clean and 110 contaminated seed were planted in light soil near a hawthorn hedge; the soil moisture was 12.92% one week after planting on 27 Apr., but thereafter and until 29 Sept., when the crop was lifted, the moisture ranged from 2.17 to 11.28%. Despite these dry soil conditions the percentage of crop tubers heavily contaminated with black scurf from the clean and contaminated seed plots respectively was 37 and 43.

I am indebted to Dr M. Cohen for the soil temperatures and to Dr T. W. Walker for the soil moisture determinations and pH values.

REFERENCE

SMALL, T. (1943). Black scurf and stem canker of potato (*Corticium Solani*). Field studies on the use of clean

and contaminated seed potatoes and on the contamination of crop tubers. *Ann. appl. Biol.* 30, 221.

(Received 8 November 1944)

Experiments on the control of white rot (*Sclerotium cepivorum* Berk.) in onions

By J. R. BOOER, PH.D., B.Sc., *Tilgate Horticultural Station, Crawley, Sussex*

(With 1 Text-figure)

Experiments made during 1943 and 1944 on the use of mercurous chloride (calomel) for the control of *Sclerotium cepivorum* in spring-sown onions are described. The application of 4% calomel dust to the seed drill at sowing time gave better results than seed treatment. 1 lb. of dust per 25 yd. of seed drill gave good disease control in bulb onions, and 1 lb./50 yd. may suffice for salad onions.

The relation between disease control and the effective mercury content of the treated soil is discussed.

INTRODUCTION

White rot of onions, caused by the fungus *S. cepivorum* Berk., was first recorded by Berkeley (1841) in Great Britain. It was subsequently recorded in Italy by Voglino (1903), in Argentina by Hauman-Merck (1915), in Spain by Caballero (1922), in various parts of the United States by Walker (1924) and Valleau (1925), in Holland by Van Poeteren (1928), in Egypt and Cyprus by Natrass (1931, 1933), in South Africa by du Plessis (1932), in Germany by Bremer (1934) and in various parts of Australia (Anon. 1938, 1943). The fungus may be seed-borne, as reported by Moore (1944), but is usually transmitted by infected seedlings, and distributed locally by cultivation. It overwinters in the soil as sclerotia, and as these are highly resistant to cold, the only cultural method of treating contaminated land is to withhold all hosts, which include several species of *Allium*, for a period of at least 7 years. The increased demand for bulb onions in England during wartime, and the general shortening of rotations, have served to emphasize the prevalence of the disease, the losses from which have become much greater during the last 2-3 years, especially in the onion-growing areas of Bedfordshire, Lincolnshire and Worcestershire.

Chemical methods of control have received relatively little attention, and none has been generally adopted. Du Plessis (1932) stated that immersion in a 0.1% mercuric chloride solution is sufficient to kill the sclerotia, but added that the use of mercuric chloride is impracticable as the quantity necessary for effective disinfection is harmful to the onion plants. Ogilvie & Hickman (1938) obtained satisfactory control of the disease by broadcast applications of a proprietary preparation containing 20% mercury as hydroxymercurichlorophenol at rates up to 1 oz./sq.yd. This method does not appear to have been adopted, probably on account of cost.

Booer (1945) reported that mercurous chloride (calomel) had given promising results, and the fol-

lowing is an account of the work on which that report is based.

Since this work was done, Mr N. C. Preston, B.Sc., of Harper Adams Agricultural College, Newport, Salop, has informed the author that in 1942 he carried out pot tests on the control of white rot in onions with calomel. He found that the application of calomel to the soil before sowing gave a high degree of control, but when calomel was applied to the surface of the soil after the plants had emerged, the treatment was much less effective.

FIELD TRIAL, 1943

This trial was originally designed to test a modification of the method recommended by Wright (1939) for the control of onion fly. Onion seed, var. James's Keeping, was coated with a preparation of mercurous chloride and an adhesive in such a way that 1 lb. of mercurous chloride adhered to 1 lb. of seed. The soil was a rich loam which had received generous applications of stable manure and charcoal dust over many years. The consistency was considerably stiffer than is generally regarded as good for onions, and the soil reaction was pH 7.5. The seed was sown on 2 Apr. in eight randomized rows 28 yd. long, four rows each treated and untreated, the rate of seeding being equal to 15 lb./acre at 12 in. row spacing. The plants emerged normally with no apparent damage to germination, and only a slight retardation in the treated rows.

About 1 June an entirely unexpected attack of white rot began to develop. The disease had not previously been recorded at the Station, and it was probably introduced in 1942 in purchased seedlings grown on a plot adjoining that used for the 1943 experiment. By mid-July it was evident that the disease was causing serious losses in the untreated rows, and it was decided to remove all diseased plants, sell most of the remaining plants as salad

onions, and leave sufficient healthy plants to produce bulbs. This was done between 29 July and 11 Aug., and the yields of healthy salad onions were equivalent to the following:

Untreated	5.6 ton/acre @ 12 in. row spacing
Treated	9.2 ton/acre @ 12 in. row spacing

The remaining plants were harvested as bulb onions on 27 Sept., the equivalent yields being as follows:

Untreated	1.8 ton/acre @ 12 in. row spacing
Treated	3.2 ton/acre @ 12 in. row spacing

During 1943 the disease was not uniform throughout the plot, and as the experiment had not been specially planned no attempt was made to examine the results statistically. It was, however, apparent from a comparison of adjacent rows that treatment of the seed with mercurous chloride had not given complete control of the disease, and this fact is reflected in the poor yield of bulb onions from the treated rows. No insect pest or disease other than white rot was recorded.

FIELD TRIAL, 1944

This trial was carried out on the same plot as the one in 1943. After the bulb onions had been removed, the ground was hand dug and no other crop was planted until spring 1944. No manure was applied. In view of the imperfect results given by seed treatment, it was decided to try the effect of localized soil treatment. The seed, var. James's Keeping, was sown on 31 Mar., the rate of seeding being equal to 16 lb./acre. The seed drills were opened and mercurous chloride applied at various rates. To facilitate application, a dust containing 4% calomel and 96% finely divided siliceous filler was dusted as uniformly as possible along the seed drill and roughly mixed with the soil. The seed was then sown and the drill closed. The treatments were as follows:

- Treatment A. Control
 B. 1 lb. 4% calomel dust per 100 yd. drill
 C. 1 lb. 4% calomel dust per 50 yd. drill
 D. 1 lb. 4% calomel dust per 25 yd. drill

Eight rows each 28 yd. long were divided into sixteen equal 'blocks' each consisting of two adjacent rows 7 yd. long. Each 'block' received one of the treatments, which were randomized on the Latin square pattern. The arrangement of the trial is shown in Fig. 1. in which the continuous lines denote the rows, and the broken lines show the division of the 'blocks'. Thus, each pair of adjacent 7 yd. rows received the treatment indicated by the letter between them.

The weather was exceptionally dry until mid-June when slight rain fell, and losses of seedlings from the disease then became evident. Periodical plant counts

were made and the results in Table 1 give the mean numbers of surviving plants per foot at various dates.

On 18 and 19 July all obviously diseased plants were removed and the healthy plants thinned to about 4 in. spacing, the surplus being sold as salad onions. The respective plant populations at this stage are shown in Table 2, the figures being the mean numbers of plants per foot.

The yields of salad onions are given in Table 3, the figures in column I being the yields actually recorded, and those in column II the estimated yields

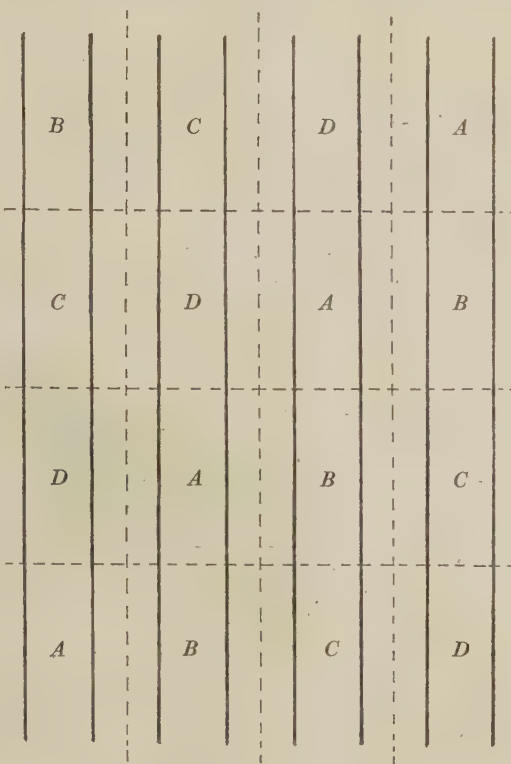


Fig. 1. Arrangement of 1944 experiment.

if all healthy plants had been pulled at this stage. The results are expressed as tons/acre on the basis of 12 in. row spacing.

The results thus far indicate that treatment C, 1 lb. of 4% calomel dust per 50 yd. of drill, would be sufficient for the needs of the grower of salad onions, and that a higher rate of application offers no advantage compatible with the additional cost.

The bulbs were lifted on 26 Sept., and cleaned, counted and weighed on 3 Oct. An analysis of the mean populations in plants per foot is given in Table 4.

TABLE 1. *Mean populations (plants/ft.) before thinning*

Date of count	Treatment			
	A	B	C	D
3 June	15	21	25	24
24 June	10	19	21	23
6 July	5	18	21	22
18 July*	2	14	19	20

* Healthy plants only.

TABLE 2. *Mean population (plants/ft.) after thinning*

	Treatment			
	A	B	C	D
Dead before thinning	19.0	6.4	2.6	3.0
Diseased thinnings	3.4	3.9	2.6	1.1
Total diseased	22.4	10.3	5.2	4.1
Healthy thinnings	0.8	10.4	15.2	16.1
Plants remaining	0.8	3.3	3.4	3.8
Total healthy	1.6	13.7	18.6	19.9
Infection %	92	43	22	17

TABLE 3. *Yields of salad onions (tons/acre)*

Treatment A	I	II
B	0.35	0.7
C	5.0	6.5
D	6.5	8.1
	7.2	8.8

TABLE 4. *Mean populations (plants/ft.) at harvest*

	Treatment			
	A	B	C	D
Died after thinning	Nil	0.7	0.5	0.3
Diseased survivors	0.3	0.9	0.5	0.5
Total diseased	0.3	1.6	1.0	0.8
Healthy bulbs	0.5	1.7	2.4	3.0
Infection %	38	49	29	21

The yields of sound onions were equivalent to the following:

Treatment A.	1.58 tons/acre
B.	4.40 tons/acre
C.	5.95 tons/acre
D.	7.90 tons/acre

These results show that the higher rate of application in treatment D, 1 lb. of 4% calomel dust per 25 yd., might be justified where bulb onions are raised from a spring sowing.

POT TESTS, 1944

At the end of the field trial, samples of soil taken as far as possible from the various drills, were examined for effective mercury content by the pot technique described by Boorer (1944). This method is based

on the fact that mercury in the soil retards the growth of wheat seedlings. The results are expressed as the interpolated mean height (*H*) of the seedlings at a time when seedlings grown in the same soil containing no mercury have reached a mean height of 6 cm. These soil samples gave the following mean results from the two closely agreeing replicates:

Treatment	<i>H</i> (cm.)	Effective Hg content
A (control)	6.00	—
B	5.98	None
C	5.78	Doubtful trace
D	5.40	Substantial amount

DISCUSSION

In the 1944 field trials, whereas treatments C and D showed little difference up to mid-July when the salad onions were harvested, the difference between the respective yields of bulb onions is more marked. This observation can be related to the results of the mercury determinations when the following additional facts are considered. Boorer (1944) has shown that mercurous chloride decomposes in the soil into metallic mercury. Daines (1936) has established that metallic mercury has fungicidal properties, and it is reasonable to suppose that a certain minimum concentration of mercury is necessary to inhibit the growth of *S. cepivorum*. Boorer has also shown that mercury in the soil is slowly converted into mercuric sulphide, which is not fungicidal. It therefore follows that a single initial application of mercurous chloride may not necessarily maintain an adequate concentration of mercury throughout the whole life of the onion plant.

In the 1943 trial the amount of mercurous chloride applied with the seed was equivalent to 12.8 lb. of mercury per acre. This treatment was, however, severely localized, and it is possible that the necessary minimum concentration of effective mercury was not maintained at the most vulnerable part of the plant, namely, that at the surface of the soil. This defect of application appears to have been largely corrected in the 1944 trials.

Treatment B is equivalent to the application of 5 lb. of mercury per acre. The results show that in this treatment the effective mercury concentration probably fell below the required minimum at an early stage of the experiment, due to the formation of mercuric sulphide.

Treatment C is equivalent to the application of 10 lb. of mercury per acre. In this case an effective concentration appears to have been maintained up to the time of harvesting the salad onions, and afterwards diminished sufficiently to allow the disease to depress the yield of bulbs.

Treatment D is equivalent to the application of 20 lb. of mercury per acre, which has been sufficient to maintain an effective concentration throughout the trial.

The treatments found by Ogilvie & Hickman (1938) to be effective were equivalent to the application of 45 and 60 lb. of mercury per acre. They used broadcast application, however, and it is of interest that they found a dosage equivalent to 15 lb. of mercury per acre had little effect. The compound they used, hydroxymercurichlorophenol, has been found by the writer to decompose fairly quickly in soil, first into calomel, and thence into metallic mercury. Consequently, their work was in reality a study of the effect of a broadcast application of mercurous chloride. As my 1944 trials clearly show,

the localized soil treatment is much more efficient than broadcast application at equal dosages of mercury.

The author gratefully acknowledges the assistance of Mr W. C. Moore, M.A., Ministry of Agriculture Plant Pathology Laboratory, Harpenden, in supplying many of the references, the permission from Mr N. C. Preston, B.Sc., Harper Adams Agricultural College, Newport, Salop, to refer to unpublished correspondence, and the help of Mr F. M. Wyatt in recording the field trials.

REFERENCES

- ANON. (1938). Plant diseases. Notes contributed by the Biological Branch. *Agric. Gaz. N.S.W.* **49**, 423.
 — (1943). White rot of onions. *J. Dep. Agric. Vict.* **41**, 312.
 BERKELEY, M. J. (1841). Notices of British fungi. *Ann. Mag. nat. Hist.* **6**, 355.
 BOOER, J. R. (1944). The behaviour of mercury compounds in soil. *Ann. appl. Biol.* **31**, 340.
 — (1945). Control of white rot in onions. *Nature, Lond.*, **155**, 241.
 BREMER, H. (1934). Die Mehlkrankheit der Zwiebeln (*Sclerotium cepivorum* Berk.). *NachrBl. dtsh. PflSch-Dienst.* **14**, 37.
 CABALLERO, A. (1922). El bioxato, o enfermedad de los ajos, en Banolas. *Bol. Soc. esp. Hist. nat.* **22**, 210. (Abstr. *Rev. appl. Mycol.* **2** (1923), 191.)
 DAINÉS, R. H. (1936). Fungicidal action of mercury in soils. *Phytopathology*, **26**, 90.
 HAUMAN-MERCK, L. (1915). Les parasites végétaux des plantes cultivées en Argentine. *Zbl. Bakt.* **43**, 447.
 MOORE, W. C. (1944). *Report on fungus, bacterial and other diseases of crops in England and Wales for the years 1933-42.* 50 pp. London: H.M. Stationery Office.
 NATTRASS, R. M. (1931). The occurrence of the 'white rot' of the onion (*Sclerotium cepivorum* Berk.) in Egypt. *Bull. Minist. Agric. Egypt*, no. 107. (Abstr. *Rev. appl. Mycol.* **11** (1932), 219.)
 — (1933). The white rot disease of onions in Cyprus. *Cyprus agric. J.* **28**, 98. (Abstr. *Rev. appl. Mycol.* **13** (1934), 348.)
 OGILVIE, L. & HICKMAN, C. J. (1938). Progress report on vegetable diseases. IX. *Rep. agric. hort. Res. Sta. Bristol for 1937*, p. 96.
 PLESSIS, S. J. DU (1932). White mould in onions. *Fmg S. Afr.* **7**, 112.
 POETEREN, N. VAN (1928). Verslag...in het jaar 1926. *Versl. PlZiekt. Dienst Wageningen*, no. 51, p. 20.
 VALLEAU, W. D. (1925). White rot of onion reported from Kentucky. *Plant Dis. Rep.* **9**, 46.
 VOGLINO, P. (1903). Sul parassitismo e lo sviluppo dell' *Sclerotium cepivorum* Berk. nell' *Allium sativum* L. *Staz. sper. agr. ital.* **36**, 89. (Abstr. *Z. PflKrankh.* **14** (1904), 110.)
 WALKER, J. C. (1924). White rot of *Allium* in Europe and America. *Phytopathology*, **14**, 315.
 WRIGHT, D. W. (1939). The control of onion fly (*Delia hylemyia antiqua* Meig.). *J. Minist. Agric.* **46**, 147.

(Received 16 February 1945)

Glitoxin, a fungistatic metabolic product of *Trichoderma viride*

By P. W. BRIAN AND H. G. HEMMING

Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks

(With 1 Text-figure)

Glitoxin is shown to be a metabolic product of *Trichoderma viride*, and a semi-continuous apparatus for its production is described. Ammonia nitrogen is preferable to nitrate nitrogen, but a wide range of carbon sources, and organic or inorganic sulphur sources, are suitable for glitoxin production. No organic supplements to a glucose-mineral medium have been found to affect glitoxin production beneficially.

Data are presented showing glitoxin to be moderately toxic to a wide range of bacteria, actinomycetes and fungi. *T. viride* itself is resistant to its toxic effects. It shows fungicidal activity when applied as a dust to cereal seeds bearing various seed-borne diseases, but is inferior to organo-mercury compounds for this purpose. Its value as a fungicide for control of plant or animal infections is reduced by the instability of aqueous solutions, except at low pH.

INTRODUCTION

The first suggestion that *Trichoderma viride* might produce substances toxic to other fungi was the observation by Falck (1931) that wood infected by *T. lignorum* (= *T. viride*) is resistant to decay by *Coniophora cerebella* and *Merulius lacrymans*. He states that the 'enzymes' of *Trichoderma viride* are toxic to those wood-destroying fungi. Weindling (1932) next recorded an antagonism between *T. viride* and certain phytopathogenic soil fungi, such as *Phytophthora* spp., *Pythium* spp. and *Corticium solani*, and noted that the antagonism is apparently due to a diffusible toxic substance produced by the *Trichoderma*. In the years that have elapsed since Weindling's observation, many workers have confirmed the antagonism between *Trichoderma* and other fungi, both *in vitro* and in soil. Weindling (1934) proceeded further with the investigation and found that if *Trichoderma* be grown on liquid media, the medium becomes markedly toxic to *Rhizoctonia* and other fungi. Weindling & Emerson (1936) isolated a crystalline substance from culture filtrates of one of their moulds; this material was subsequently named (Weindling, 1941) glitoxin, as Weindling (1937) had in the meantime amended the identification of the mould concerned to *Gliocladium fimbriatum*. It has been shown since (Brian, 1944) that the mould from which Weindling isolated glitoxin was, after all, probably a strain of *Trichoderma viride*, and that strains of *T. viride* do, in fact, produce glitoxin.

Glitoxin has been shown (Johnson *et al.* 1943, 1944) to be a substance of rather unusual chemical structure, having the molecular formula $C_{13}H_{14}N_2S_2O_4$. It has since been recorded as a metabolic product of the moulds *Aspergillus fumigatus* (Menzel *et al.* 1944; Glistler & Williams, 1944) and an incompletely identified *Penicillium* sp. (Johnson *et al.* 1944).

The present investigation deals with the conditions under which glitoxin is produced by two strains of *Trichoderma viride*, one of which is believed to be identical with the organism used by Weindling under the name *Gliocladium fimbriatum*, and gives further information on the biological properties of glitoxin.

EXPERIMENTAL MATERIALS AND METHODS

(a) Glitoxin-producing strains of *Trichoderma viride*

Two strains of *Trichoderma viride* have been used in this investigation, viz.:

Strain no. 12. Originally received from Prof. H. Raistrick in October 1942, as *Gliocladium fimbriatum*, 'Weindling's strain', since shown (Brian, 1944) to be a strain of *Trichoderma viride*.

Strain no. 211. Isolated in March 1944, from a greenhouse soil at Jealott's Hill.

Both these strains are typical of *T. viride* in size of conidia, presence of chlamydospores, and rapid growth. They are to some extent aberrant in that the spore balls are not borne on one or two phialides arising directly from fertile hyphae, as is usually the case in *Trichoderma*, but a proportion are borne on groups of 6-8 phialides arising from a terminal verticil of metulae or branches. The presence of this more complex, rather *Gliocladium*-like conidiophore appears to be associated with a diffuse habit of growth. Those strains of *Trichoderma viride* which form compact tufts usually have the simple type of conidiophore; the complex type characteristic of the strains mentioned above, though concentrated to some extent into tufts, is usually distributed over the whole of the aerial surface of the growing colony. The two strains differ slightly in their macroscopic

appearance on various culture media, and, as will be shown later, in their capacity to produce gliotoxin.

For production of spores Czapek-Dox agar has been found most satisfactory.

(b) Methods of assay

Bacteriostatic potency of culture filtrates has been measured by a serial dilution technique similar to that described by many other workers.

For assay of fungistatic potency a spore-germination technique has proved very satisfactory, and for routine work is more accurate than serial dilution bacteriostatic assays based on visual opacity determinations. It is thought worth while to describe this method in some detail.

Preparation of standard spore suspension. For normal purposes the conidia of *Botrytis allii* Munn. are used. Czapek-Dox tube cultures, grown 7 days at 25°C., are washed with germination medium (see below), and fragments of agar and mycelium removed by filtration through sterile cotton gauze. The spore density of the suspension is standardized, using a haemocytometer, to 0.5 million spores per ml.

Dilutions. For each sample to be assayed the procedure is as follows:

(a) Serial dilutions of the solution to be assayed are made with germination medium (dextrose 12.5 g., ammonium tartrate 1.0 g., potassium dihydrogen phosphate 1.0 g., magnesium sulphate 0.5 g., distilled water 1000 ml.), 2 ml. of the test solution being added to 2 ml. of germination medium, 2 ml. of this transferred to a further 2 ml. of germination medium, and so on until sufficient dilutions have been made. Precautions to ensure sterility are undertaken throughout.

(b) To each 2 ml. of diluted test solution prepared as above, 2 ml. of spore suspension are added. Thus, the first dilution is a 1 in 4 dilution of the original test solution, the next 1 in 8 and so on.

Incubation. With a sterile dropping pipette three drops of each dilution, containing the *B. allii* spores, are placed on a microscope slide, which is placed in a Petri dish moist chamber and incubated for 16–18 hr. at 25°C.

Reading results. With many solutions being assayed at the same time, it would be impossible to make a detailed germination count. The drops are therefore examined under the low power of a microscope, and the degree of germination estimated at a glance as belonging to one of the following categories:

- 0 : No germination.
- (1) : Less than 1 % germination.
- 1 : 1–5 % germination.
- 2 : 5–50 % germination.
- 3 : 50–90 % germination.
- 4 : Complete (90–100 %) germination.

This can be done very rapidly with a little practice.

Usually all three drops on a slide give identical results. When they do not, a mean value is taken.

The fungistatic activity of a given solution can then be expressed in units ('BA units'), the number of units of activity per ml. being the number of times the original solution can be diluted, in the germination drops, and still prevent germination of the *B. allii* spores. For this purpose grade (1) is taken to be the same as grade 0.

Inspection of the data given in Table 1 will show how closely this assay follows the actual production of gliotoxin.

(c) Methods of culture

For experiments where the effect of various culture conditions on the antibiotic activity of the culture filtrate is being studied, without extraction of the active material, cultures on 30 ml. of liquid medium in 100 ml. conical Pyrex flasks have been found convenient. The contents of five such flasks are bulked before assay to minimize differences due to variations in individual conditions in the flasks. Care

TABLE 1. *Production of gliotoxin by continuous flow process (Trichoderma viride, strain 12)*

Weeks growth	Vol. of filtrate collected ml.	Total weekly activity BA units $\times 10^3$	Yield of gliotoxin mg.
1	19,620	30,660	1520
2	23,180	31,182	1503
3	25,050	38,348	1396
4	23,360	12,423	621
5	22,560	9,244	312

is taken in such experiments that all flasks receive equal inocula and are distributed in random fashion in the incubator room.

Where cultures for production of gliotoxin are required, flat earthenware vessels, as used by Abraham *et al.* (1941) for production of penicillin, have been used. Culture filtrates of maximum potency are produced by using thin layers of medium (250 ml. per vessel), and incubating the cultures for 4 days at 25°C. Although *Trichoderma viride* is a rapidly growing mould, it is remarkable that appreciable concentrations of gliotoxin are produced in so short a time. The medium used has been that originally proposed by Weindling (1934), viz. dextrose 25.0 g., ammonium tartrate 2.0 g., potassium dihydrogen phosphate 2.0 g., magnesium sulphate 1.0 g., ferrous sulphate 0.01 g., distilled water 1000 ml., adjusted to pH 3.5 with phosphoric acid before autoclaving. On this medium the fungus grows well, produces maximum quantities of gliotoxin, but produces no spores at all, thus avoiding wholesale contamination of the laboratory atmosphere during the extraction procedure. If the medium is adjusted to pH 6.5, sporulation is abundant, but production of gliotoxin little affected.

This method, though reliable, is rather troublesome to carry out, involving much handling of culture vessels and the occupation of much incubator space. The development of a simple continuous process, similar to that described by Clifton (1943), has been found valuable. The mould is grown on a substratum of folded blotting paper, supported in a glass or earthenware tower. Sterile medium (Weindling) is dropped continuously over the growing mycelial felt, a current of sterile air blown through, and the medium issuing from the base of the column collected for extraction. A successful apparatus utilized an earthenware tower 14 in. high and of 7 in. internal diameter, provided with a tightly fitting lid and containing five fluted blotting paper cylinders, arranged as shown in Fig. 1. Sterile medium was dropped

(d) *Extraction of gliotoxin from culture filtrates*

The culture filtrate is extracted three times with one-tenth of its volume of chloroform. The chloroform is then evaporated at reduced pressure to a small volume, and an excess of petrol ether (B.P. 60–80°C.) added. The gliotoxin is precipitated out, with a certain amount of gummy, coloured material. This crude gliotoxin may be purified by washing with a little warm ethanol, followed by recrystallization from the same solvent. It is then obtained in the form of colourless needle-shaped crystals. Typical analyses of the product from both strains of *T. viride* are given in Table 2.

It will be seen that these analyses agree with $C_{13}H_{14}N_2S_2O_4$ as found for gliotoxin by Johnson *et al.* (1943).

TABLE 2. *Analyses of gliotoxin*

%	Calc. for $C_{13}H_{14}N_2S_2O_4$	Gliotoxin from strain no. 12	Gliotoxin from strain no. 211
C	47.8	47.8	47.8
H	4.3	4.4	4.4
S	19.7	19.8	19.9
N	8.6	8.6	8.3

EFFECT OF COMPOSITION OF CULTURE MEDIUM ON
DEVELOPMENT OF FUNGISTATIC ACTIVITY

Raistrick and his colleagues have found Czapek-Dox and Raulin-Thom media to be very useful in studies of the metabolism of moulds, and have remarked that the behaviour of the mould may be very different on these two media, which differ mainly in that the

TABLE 3. *Fungistatic activity (BA units) of culture filtrates from two strains of Trichoderma viride*

Strain no.	Medium	Days' growth at 25°C.					
		4	6	8	10	12	14
12	Weindling	32	64	128	128	512	256
	Czapek-Dox	—	—	64	128	128	4
	Corn-steep	—	—	64	128	—	4
	Raulin-Thom	—	8	32	64	256	128
	Weindling	16	32	512	1024	2048	1024
211	Czapek-Dox	4	4	64	64	16	32
	Corn-steep	—	4	8	32	16	16
	Raulin-Thom	8	256	1024	512	1024	1024

from four jets, and filtered air blown through. The medium was dropped through slowly for the first 3 days, while a mycelial felt was being established, and then the rate increased to about 3 l. per day. Production of gliotoxin then maintained a steady rate for 3 weeks (see Table 1), after which there was a gradual fall. The fall was attributed to the formation of a thick cap of mycelium at the top of the blotting paper cylinders which tended to divert the flow of medium from the mycelial felt to the sides of the vessel. Little trouble with contaminations of any kind has been experienced, and using the blotting paper substratum fewer blockages are experienced than is the case when the wood chip substratum recommended by Clifton (1943) is used. The apparatus is quite compact; in 3 weeks it produces a quantity of gliotoxin which, by the still culture method, would entail a far greater expenditure of labour. The culture filtrate dripping through the tower frequently contains a higher concentration of gliotoxin than can be obtained in still culture.

former has sodium nitrate as nitrogen source, the latter various ammonium salts. More recently, Waksman & Horning (1943) have shown that addition of corn-steep liquor, a by-product of the starch industry, markedly increases the production of penicillin by some strains of *Penicillium notatum*. Accordingly, these media have been compared with Weindling medium for production of fungistatic culture filtrates by *Trichoderma viride*. Results are shown in Table 3. The formula of Weindling medium has already been given, Czapek-Dox and Raulin-Thom media have been made up as recommended by Raistrick (1931), and the corn-steep medium is essentially a Czapek-Dox medium, containing 2% glucose and 2% lactose as carbon source, with 8% added corn-steep liquor.

It will be seen that with both strains of the mould, Raulin-Thom and Weindling media produce culture filtrates of greatest activity, and this activity is maintained for a longer period than with the other two media. The fall in activity after the 10th day, with

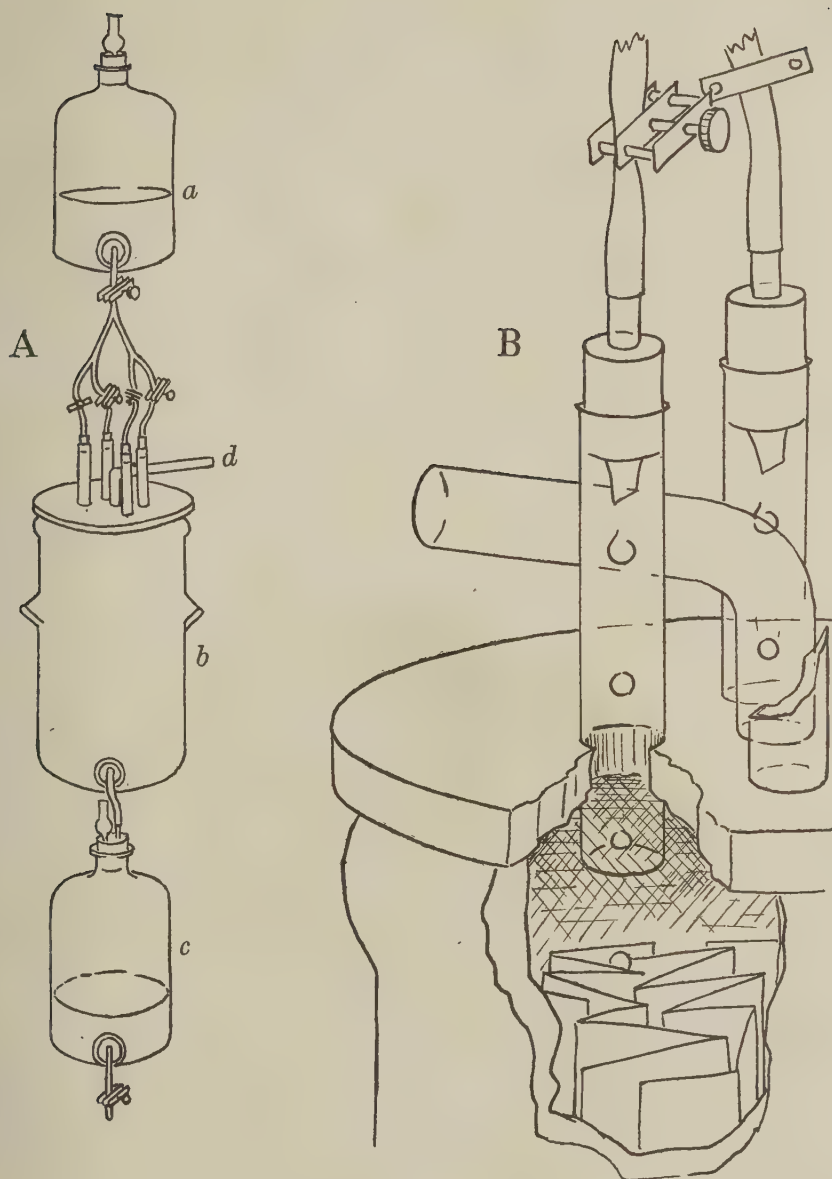


Fig. 1. A. General view of semi-continuous apparatus for production of gliotoxin, showing (a) medium reservoir, (b) earthenware culture-tower, (c) collecting vessel and (d) air-inlet. B. Portion of upper part of culture-tower enlarged to show arrangement of jets and folded paper culture-substratum.

Czapek-Dox and corn-steep media, is believed to be due to the high pH developed on these media (presumably due to selective absorption of phosphate and nitrate ions); as will be shown later, gliotoxin solutions lose activity rapidly at high pH. Strain no. 211 produces filtrates of higher activity than no. 12; this is confirmed by the fact that on an average twice as much gliotoxin is produced, about 100 mg./l. being produced by strain no. 211 under conditions where from strain no. 12 only about 50 mg. are obtained. The superiority of the Weindling and Raulin-Thom media is not due entirely to the maintenance of acidity, as other experiments have shown that in media buffered at pH 3.5, the ammonium salts are superior to nitrates, and of various ammonium salts tried, the citrate, phosphate, sulphate and tartrate give equally good results, the chloride being rather inferior.

TABLE 4. *Effect of varying carbon source in Weindling medium on fungistatic activity of Trichoderma viride culture filtrates*

Carbon source	Degree of growth	BA units	
		4 days' growth	10 days' growth
Glucose	+++	340	85
Galactose	++	340	680
Maltose	++	21	340
Lactose	++	340	340
Mannitol	+++	340	340
Dulcitol	++	21	340
Salicin	++	21	5
Dextrin	+++	85	85
Starch	++	340	680
Glycerol	++	340	340
Ethanol	+	85	340
Citrate	+	<5	<5
Tartrate	+++	<5	<5

A wide variety of carbon sources can be utilized by *T. viride* for growth and for production of gliotoxin, but these two functions are not necessarily correlated (Table 4). Most of these carbon sources were of the same order as glucose, both for growth and for production of fungistatic activity in the culture filtrate. There were some interesting exceptions. Ethanol was a very poor carbon source for growth, but considerable fungistatic activity developed; citrate and tartrate were very poor carbon sources from the point of view of activity of the culture filtrate, though tartrate supported very good growth.

As gliotoxin contains sulphur it was thought that the nature of the sulphur supply in the medium might affect gliotoxin production. However, it appears that sulphur may be supplied as sulphate or in organic form as cystine or cysteine equally suitably.

A wide variety of supplements to the medium, such as Difco yeast extract, Marmite, peptone, glycine, thiamin, potato extract and malt extract,

have been found on occasion to give slightly better growth, but never appreciably to affect the production of gliotoxin. An interesting exception is found in corn-steep liquor which, in concentrations between 1 and 5% in the medium, noticeably increases the amount of mycelium formed, but almost completely inhibits gliotoxin production.

BIOLOGICAL ACTIVITY OF GLIOTOXIN

Comprehensive data on the toxicity of gliotoxin to various bacteria and fungi have been given by Johnson *et al.* (1943) and by Waksman & Woodruff (1942). Results given in Table 5 extend and confirm these data; mercuric chloride and acriflavine have been included as standards of fungistatic and bacteriostatic activity respectively.

In interpreting the figures given in Table 5, the different techniques used in different cases should be noted. The data relating to bacteria have been obtained by serial dilutions in peptone-Lemco broth. In the case of the pathogenic actinomycetes and dermatophytic fungi, the gliotoxin was included in tubed agar media of suitable composition for the growth of the organisms, Waksman's glucose-asparagine agar for the actinomycetes (Waksman, 1931), and Czapek-Dox agar with 0.1% Difco yeast extract for the dermatophytes. Activity against the remaining fungi was determined by the spore germination test already described. Experience has indicated that concentrations of a toxic substance which prevent germination are frequently lower than those required to prevent growth in agar media. The general impression obtained from these results is of moderate all-round toxicity. The extreme resistance of *T. viride*, which produces gliotoxin, is very noticeable.

Whereas *Botrytis allii* spores will not germinate in the presence of concentrations of 3.75 µg./ml. of gliotoxin, much higher concentrations are needed actually to kill the spores. It has been found that if *B. allii* spores are exposed for 15 min. to aqueous solutions of gliotoxin of concentrations of 80 µg./ml. or over, the spores will not germinate even if washed several times in sterile buffer (pH 3.5). If the gliotoxin concentration is 40 µg./ml. or less, the spores will germinate if washed twice in sterile buffer and centrifuged out before placing in the germination medium. Thus the concentration of gliotoxin required to kill *B. allii* spores in 15 min. is about 20 times greater than that which prevents germination if present in the germination medium.

The possible value of gliotoxin for control of seed-borne diseases of cereals has been investigated. Dressings containing proportions varying from 5 to 100% of gliotoxin were applied to oats, barley and wheat seed known to carry various seed-borne infections, at a rate of 2 oz./bushel. These were sown in rod-rows out of doors in the autumn of 1943 and disease and emergence counts were made at appro-

priate times. The results, with a comparison with a proprietary organo-mercurial dressing, are given in Table 6. The higher concentrations of gliotoxin cause marked disease reduction, though much inferior to the organo-mercurial. No phytocidal effects were observed.

The solutions of gliotoxin (100 µg./ml.) were made in sterile citric acid-phosphate buffer (McIlwaine's). At pH 3.5, gliotoxin solutions are stable; indeed at this pH they can be autoclaved with little loss of activity. At higher pH, even though still appreciably on the acid side of neutrality (e.g. pH 5.4), loss of

TABLE 5. *Toxicity of gliotoxin to various organisms*

Organism	Least conc. (µg./ml.) inhibiting growth		
	Gliotoxin	HgCl ₂	Acridine
Bacteria:			
* <i>Bacillus subtilis</i> (N.C.T.C. 3610)	10.0	—	3.9
* <i>Bacterium lactis aerogenes</i> (N.C.T.C. 4140)	80.0	—	250.0
* <i>Escherichia coli</i> (N.C.T.C. 4144)	20.0	—	62.5
* <i>Micrococcus lysodeikticus</i> (N.C.T.C. 2665)	0.15	—	0.5
* <i>Salmonella typhi</i> (N.C.T.C. 786)	10.0	—	7.8
* <i>Staphylococcus aureus</i> (N.C.T.C. 3750)	3.0	—	2.0
Actinomycetes:			
† <i>Actinomyces beddardi</i> (N.C.T.C. 4579)	20.0	20.0	—
† <i>A. pretorianus</i> (N.C.T.C. 2391)	40.0	20.0	—
† <i>A. willmorei</i> (N.C.T.C. 1856)	40.0	20.0	—
Fungi:			
† <i>Aspergillus niger</i>	30.0	0.3	—
† <i>Bodinea violacea</i> (N.C.T.C. 2523)	20.0	10.0	—
† <i>Botrytis allii</i>	3.75	0.3	—
† <i>Colletotrichum lini</i>	0.9	0.3	—
† <i>Endomycopsis albicans</i> (N.C.T.C. 714)	80.0	20.0	—
† <i>Fusarium caeruleum</i>	1.9	0.18	—
† <i>F. culmorum</i>	3.75	0.18	—
† <i>Macrosporium sarcinaeforme</i>	7.5	0.3	—
† <i>Penicillium digitatum</i>	30.0	3.0	—
† <i>P. expansum</i>	15.0	0.18	—
† <i>Sabouraudites audouinii</i> (N.C.T.C. 2526)	10.0	5.0	—
† <i>Sporotrichum beurmannii</i> (N.C.T.C. 3017)	80.0	5.0	—
† <i>Trichoderma viride</i> (No. 12)	> 160.0	0.3	—
† <i>Trichophyton tonsurans</i> (N.C.T.C. 2520)	20.0	10.0	—
† <i>Trichothecium roseum</i>	3.75	0.3	—

* Gliotoxin in serial dilutions in peptone-Lemco broth.

† Gliotoxin included in agar media.

‡ Gliotoxin in serial dilutions in germination medium, assayed by germination test.

TABLE 6. *Control of seed-borne diseases of cereals by gliotoxin*

Treatment	Leaf spot of oats (<i>Helminthosporium avenae</i>)		Covered smut of barley (<i>Ustilago hordei</i>)		Bunt of wheat (<i>Tilletia caries</i>)	
	Disease %	Emergence %	Disease %	Emergence %	Disease %	Emergence %
100% gliotoxin	5.4	82.1	0.7	87.8	9.8	84.7
50% gliotoxin on talc	6.5	73.9	1.1	90.3	9.2	87.9
10% gliotoxin on talc	9.6	74.6	1.5	84.3	14.9	83.1
5% gliotoxin on talc	10.1	74.8	0.5	87.7	16.6	82.4
Agrosan G	0.2	82.4	0.5	87.4	0.5	88.2
Untreated	15.6	73.8	3.5	84.6	29.1	75.5

STABILITY OF AQUEOUS SOLUTIONS OF GLIOTOXIN IN RELATION TO pH

Weindling (1941) has presented data showing the instability of gliotoxin in alkaline aqueous solutions. The data in Table 7 give a somewhat more complete picture of the relation between pH and its stability.

activity occurs. The rate of decomposition is not, however, so great as to preclude an important natural role for gliotoxin. In most cultivated soils *Trichoderma viride* is a major constituent of the soil microflora. In soils of average acidity the rate of decomposition of gliotoxin is not likely to exceed by much

the rates of production, so that in the immediate vicinity of *Trichoderma* hyphae, at least, a profound influence on the microflora might be expected. In

TABLE 7. *Effect of pH on fungistatic activity of aqueous solutions of gliotoxin (100 µg./ml.) maintained at 25° C.*

Days' storage	Activity in BA units				
	pH 3.4	pH 5.4	pH 6.6	pH 7.5	pH 8.2
0	32	32	32	32	32
1	32	32	16	16	—
3	32	16	4	4	—
4	16	16	2	—	—
7	32	8	—	—	—
9	16	2	—	—	—
14	16	—	—	—	—

more highly acid soils it seems possible that gliotoxin might even tend to accumulate, resulting in much decreased microbiological activity.

The possibility of using gliotoxin as a fungicide

for control of plant or animal infections can be considered in the light of these results. For application to animals the fairly rapid loss in activity near neutrality is a considerable disadvantage; its marked toxicity to animals (Johnson *et al.* 1943) must also be taken into consideration. Though it would seem to have limited prospects for application to animals, it is more likely to find a use as a plant fungicide, where acid formulations might be used. Even in this field, where a lasting protectant effect is required, its value seems doubtful. Any such development also depends on the development of a method of synthesis that would render its production economic.

We are indebted to Mr C. Campbell for the preparation of Fig. 1, to Mr J. C. McGowan for the chemical identification of gliotoxin and to Mr R. V. Tipler for examination of gliotoxin as a cereal seed treatment. We wish to thank Dr R. C. Woodward for his advice and encouragement during the progress of this investigation.

REFERENCES

- ABRAHAM, E. P., CHAIN, E., FLETCHER, C. M., GARDNER, A. D., HEATLEY, N. G., JENNINGS, M. A. & FLOREY, H. W. (1941). Further observations on penicillin. *Lancet*, no. 241, pp. 177-88.
- BRIAN, P. W. (1944). Production of gliotoxin by *Trichoderma viride*. *Nature, Lond.*, **154** (3917), 667.
- CLIFTON, C. E. (1943). Large-scale production of penicillin. *Science*, **98**, 69.
- FALCK, R. (1931). Ueber den Einfluss des Flössens auf die Widerstandsfähigkeit des Bauholzes gegen Trockenfäule und über den Holzschutz durch Schimmelbefall und Diffusionsstränkung. *Mitt. Forstwiss. Forstwiss.* pp. 480-5.
- GLISTER, G. A. & WILLIAMS, T. L. (1944). Production of gliotoxin by *Aspergillus fumigatus* mut. *helvola* Yuill. *Nature, Lond.*, **153** (3891), 651.
- JOHNSON, J. R., BRUCE, W. F. & DUTCHER, J. D. (1943). Gliotoxin, the antibiotic principle of *Gliocladium fimbriatum*. I. Production, physical and biological properties. *J. Amer. chem. Soc.* **65**, 2005.
- JOHNSON, J. R., MCCRONE, W. C. & BRUCE, W. F. (1944). Gliotoxin, the antibiotic principle of *Gliocladium fimbriatum*. II. *J. Amer. chem. Soc.* **66**, 501.
- MENZEL, A. E. O., WINTERSTEINER, O. & HOOGERHEIDE, J. C. (1944). The isolation of gliotoxin and fumigatin from culture filtrates of *Aspergillus fumigatus*. *J. biol. Chem.* **152**, 419-29.
- RAISTRICK, H. (1931). Studies in the biochemistry of microorganisms. *Philos. Trans. B*, **220**.
- WAKSMAN, S. A. (1931). *Principles of Soil Microbiology*, 2nd ed. London: Baillière, Tindall and Cox.
- WAKSMAN, S. A. & HORNING, E. S. (1943). Distribution of antagonistic fungi in nature and their antibiotic action. *Mycologia*, **35** (1), 47-65.
- WAKSMAN, S. A. & WOODRUFF, H. B. (1942). Selective antibiotic action of various substances of microbial origin. *J. Bact.* **44** (3), 373-84.
- WEINDLING, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*, **22** (10), 837-45.
- (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology*, **24**, 1153-79.
- (1937). Isolation of toxic substances from the culture filtrates of *Trichoderma* and *Gliocladium*. *Phytopathology*, **27**, 1175-7.
- (1941). Experimental consideration of the mold toxins of *Gliocladium* and *Trichoderma*. *Phytopathology*, **31**, 991-1003.
- WEINDLING, R. & EMERSON, O. H. (1936). The isolation of a toxic substance from the culture filtrate of *Trichoderma*. *Phytopathology*, **26**, 1068-70.

(Received 5 January 1945)

Studies in the dynamics of disinfection

IV. The reaction between phenol and *Bact. coli*: the true shape of the probit-log survival-time curve

BY R. C. JORDAN, PH.D.

Physiology Institute, University College of South Wales and Monmouthshire, Cardiff

AND S. E. JACOBS, PH.D.

The Bacteriological Laboratory, Imperial College of Science and Technology, London

(With 7 Text-figures)

The results of numerous experiments on the disinfection of standard cultures of *Bact. coli* with phenol, under rigidly controlled conditions, have been combined and used to elucidate the true shape of the probit-log survival-time curve. It is concluded that the true shape is that of a very asymmetrical sigmoid curve. When the disinfection is slow, the curve approximates closely to a bilinear form with one line of small and one of large slope up to high probit values, but in faster disinfections the curvature of the steeper line is evident, especially above the probit value of 8.

The fact that the survivors-time curves for bacteria exposed to lethal agencies are of somewhat unusual type has led to considerable speculation concerning the underlying causes. The literature on the subject has been reviewed by Chick (1930) and by Rahn (1932, 1943). Broadly speaking, there are two points of view, one being that the individual bacterial cells of a culture are essentially similar and, accordingly, the shape of the survivors-time curve is regarded as being due to the nature of the reaction actually causing death. The other view is that the cells have a graded susceptibility, and that the shape of the survivors curve depends primarily on that fact. Objections which are difficult to meet have been raised against each view, but it is not improbable that both factors are operative. More recently, Withell (1942) has used the probit method of Bliss (1935) in an examination of the distribution of survival times amongst the cells of a bacterial population exposed to lethal agents. He showed that, when the resistance of an organism is considered to be proportional to the logarithm of its survival time, the distribution of resistance among the population approximates to that given by the normal frequency curve. Jordan & Jacobs (1944*a*) obtained further evidence in support of this conclusion, but at that time it was not examined critically.

It should be stressed that the term 'resistance', as used here in its customary bacteriological sense, is a composite one conveniently embracing all the factors which together enable any individual cell to retain its viability in adverse conditions for a longer or a shorter time, and which are not necessarily inherent in the organism itself. When the probit

method is employed it is with the object of demonstrating that this so-called resistance, when expressed as some function of the survival time, is normally distributed, but no assumptions or theoretical considerations regarding the intimate nature of the resistance are involved. All that the method does is to afford a convenient means of detecting the particular function of survival time which must be used for the realization of the normal distribution. The great value of the method from the practical point of view is that the probit graph which demonstrates the existence of a normal distribution of resistance is linear and forms a convenient simplification of a complicated sigmoid survivors-time curve. From any set of data the best-fitting straight line can easily be calculated and any desired mortality time, with its standard error, derived from it. Accordingly, it is important that the evidence for the statement that probit-log survival-time graphs are linear for bacterial cultures exposed to lethal agencies should be examined critically. A considerable number of experiments having been performed on the disinfection of standard cultures of *Bact. coli* by phenol under rigidly controlled conditions (Jordan & Jacobs, 1944*a*, 1945), it was decided to analyse the results with this end in view.

RESULTS AND DISCUSSION

The probit-log survival-time data derived from a number of experiments on the disinfection of *Bact. coli* cultures in which several temperatures at each of five phenol concentrations were used (Jordan & Jacobs, 1945) are given in Table 1, and, together

Phenol conc. g./l.	Temp. °C.	Time min.	Log ₁₀ time	Probit	Slope of probit- log time line
--------------------------	--------------	--------------	---------------------------	--------	---

Temperatures				Slope of		6.01		31		15		1.761		4.5109		—	
Phenol	Temp.	Time	Log ₁₀	Probit	probit-	log	time	line									
conc.	°C.	min.	time														
g./l.																	
7.95	29	5	0.6990	4.7136	—												
		25	1.3979	5.7784	1.52												
		45	1.6532	8.6367	11.20												
		65	1.8129	9.2440	3.80												
		80	1.9031	9.7836	5.98												
		95	1.9777	10.0881	4.08					28	5	0.6990	3.6740	—			
		110	2.0414	10.3538	4.17						60	1.7782	4.2031	0.49			
											90	1.9542	4.3187	0.66			
	24	10	1.0000	4.5405	—						125	2.0969	5.3401	0.15			
		45	1.6532	5.1611	0.95						195	2.2900	7.4568	10.95			
		80	1.9031	6.4857	5.30						240	2.3802	8.4771	11.31			
		115	2.0607	7.8099	8.40						275	2.4393	9.0146	9.09			
		160	2.2041	9.3000	10.40						305	2.4843	9.1200	2.34			
		195	2.2900	9.8218	6.07						345	2.5378	9.2050	1.59			
		230	2.3617	9.9025	1.13						375	2.5740	9.4350	6.35			
		265	2.4232	10.1602	4.18												
20	10	1.0000	4.1686	—					26	5	0.6990	3.3268	—				
		45	1.6532	4.5961	0.65					90	1.9542	4.3415	0.81				
		85	1.9294	4.7642	0.61					185	2.2672	5.2107	2.78				
		125	2.0969	5.8796	6.66					270	2.4314	7.0642	11.28				
		175	2.2430	6.7716	6.10					360	2.5563	8.2757	9.72				
		220	2.3424	7.5679	8.02					535	2.7284	9.4590	6.88				
		260	2.4150	8.4083	11.60					615	2.7889	10.0494	9.76				
		305	2.4843	8.8270	6.04					690	2.8388	10.3575	6.17				
	30.5	350	2.5441	9.6026	13.00					23	1040	3.0170	9.5839	—			
		395	2.5966	9.9506	6.63						1115	3.0473	9.6768	3.06			
		440	2.6435	10.2186	5.72						1190	3.0756	9.9968	11.30			
											1270	3.1038	10.1989	7.17			
		5	0.6990	4.5025	—						1340	3.1271	10.2739	3.22			
		35	1.5441	6.1988	2.01						1400	3.1461	10.4564	9.60			
		65	1.8129	8.5852	8.88						1655	3.2188	10.8150	4.93			
		110	2.0414	9.5278	4.12					5.20	39	5	0.6990	4.6209	—		
6.98	28	120	2.0792	9.7691	6.38					35	1.5441	6.9972	2.81				
		130	2.1139	9.9287	4.60					60	1.7782	8.8727	8.01				
		140	2.1461	10.3179	12.10					85	1.9294	9.9188	6.92				
										105	2.0212	10.2122	3.19				
		10	1.0000	4.0516	—					31	30	1.4771	3.4160	—			
		40	1.6021	5.0173	1.61						90	1.9542	4.1269	1.47			
		80	1.9031	7.5960	8.56						160	2.2041	4.3293	0.81			
		115	2.0607	8.9700	8.72						330	2.5185	6.4101	6.62			
	25	150	2.1761	9.2750	2.64						375	2.5740	7.1729	13.74			
		185	2.2672	9.7187	4.87						415	2.6180	7.9035	16.60			
		220	2.3424	10.1332	5.50						465	2.6675	8.7158	16.41			
		10	1.0000	4.3720	—						515	2.7118	9.0185	6.81			
		65	1.8129	4.9101	0.66						565	2.7520	9.1543	3.38			
		120	2.0792	6.2270	4.94					27	60	1.7782	3.5981	—			
		185	2.2672	8.1490	10.22						115	2.0607	3.8250	0.80			
		240	2.3802	8.9569	7.15						310	2.4914	4.1121	0.67			
22	22	295	2.4698	9.1022	1.62					355	2.5502	4.5600	7.62				
		345	2.5378	9.3350	3.39					415	2.6180	4.6206	0.89				
		405	2.6075	9.8960	8.04					475	2.6767	4.9049	4.84				
		450	2.6532	10.3354	9.62					535	2.7284	5.2290	6.27				
		485	2.6857	10.4991	5.03					915	2.9614	7.7059	10.63				
										1120	3.0492	9.1700	16.68				
		5	0.6990	3.8118	—						1195	3.0774	9.4700	10.64			
		65	1.8129	4.6438	0.75						1285	3.1089	9.8522	12.13			
	22	555	2.7443	9.5030	5.22						1355	3.1319	10.1335	12.23			
		625	2.7959	10.0554	10.70						1420	3.1523	10.2090	3.70			
		695	2.8420	10.2277	3.74						1480	3.1703	10.5917	21.26			
		755	2.8780	10.5055	7.72												
		830	2.9191	10.6975	4.67												

Table 1 (*continued*)

Phenol conc. g./l.	Temp. °C.	Time min.	Log ₁₀ time	Probit	Slope of probit- log time line
4.62	42	5	0.6990	4.0351	—
		25	1.3979	5.1479	1.59
		70	1.8451	9.4750	9.68
		95	1.9777	9.9734	3.31
		115	2.0607	10.5833	8.07
39.5	39.5	5	0.6990	3.7655	—
		40	1.6021	4.6031	0.93
		80	1.9031	7.1542	8.48
		165	2.2175	9.5671	7.67
		200	2.3010	9.9027	4.02
		240	2.3802	10.1254	2.81
		280	2.4472	10.6975	8.54
38	38	5	0.6990	3.8559	—
		35	1.5441	4.3668	0.60
		65	1.8129	4.5540	0.51
		95	1.9777	5.4305	5.32
		125	2.0969	6.7966	11.46
		155	2.1903	8.1473	14.46
		185	2.2672	8.9430	10.35
		215	2.3324	9.6507	10.85
		245	2.3892	10.1272	8.39
		275	2.4393	10.3718	4.88
32.5	32.5	90	1.9542	3.7572	—
		220	2.3424	4.1928	1.12
		335	2.5250	4.5942	2.20
		390	2.5911	4.8915	4.50
		580	2.7634	6.7227	10.63
		635	2.8028	7.1945	11.97
		685	2.8357	7.7612	17.22
		760	2.8808	8.2954	11.84
		840	2.9243	8.9760	15.65
		800	2.9031	5.1642	—
30	30	930	2.9685	6.0572	13.65
		1050	3.0212	6.3077	4.75
		1160	3.0645	6.8912	13.48
		1235	3.0916	7.0517	6.15
		1440	3.1584	8.0362	14.74
		1530	3.1847	8.4102	14.22
		1670	3.2227	8.7524	9.01
		2040	3.3096	10.3627	18.53

with similar data from experiments at 35°C. already published (Jordan & Jacobs, 1944*a*), are shown graphically in Figs. 1–5. Although, for reasons which will appear later, these graphs have been drawn as smooth curves there is apparently considerable justification for the statement that they approximate to a bilinear form with an abrupt transition between the two lines. There is no indication that the graphs change gradually into single straight lines when the whole disinfection is very rapid, as was previously thought might be the case (Jordan & Jacobs, 1944*a*). The impression that the graphs are bilinear is strengthened when the best-fitting straight lines are calculated, using unweighted probit values. The slopes of these lines and their standard errors are given in Table 2, which also includes certain experiments not represented in Figs. 1–5 as they were

performed at other phenol concentrations. The standard errors are, in general, sufficiently small for the fit to be considered good. It is not proposed to discuss in detail the manner in which the slopes of these lines vary with temperature and phenol concentration, since it will be shown below that this method of treating the data affords only an approximation to the truth. It is, however, of importance to realize that if it is assumed that a linear relationship between probits and log survival-time is a satisfactory representation of the results, then it must be accepted that at constant temperature the slopes of these lines vary markedly with changing phenol concentration. At 35°C. the slopes of the second-part lines fall away at high and low concentrations, and there are indications that this happens also at other temperatures though the data are less extensive. The result of this must be that when the probit lines are used to calculate various mortality times the values of the concentration exponent derived from them must vary in magnitude and in accuracy according to the degree of mortality chosen. Jordan & Jacobs (1944*b*) have already shown that at 35°C. the value of the concentration exponent for phenol does vary in this manner with the degree of mortality chosen.

It may also be pointed out that at high phenol concentrations the slopes of the second-part lines of the probit-log survival-time graphs are hardly affected by changing temperature, but there is a marked reduction in slope at lower concentrations when the temperature is high. This finding may, in part, be due to differences in the positions of the probit values in the experiments concerned. If the slopes calculated between successive determinations in each experiment (see Table 1) are examined, it will be seen that in many cases, after an initial period at low values, they suddenly rise to peaks from which they subsequently decline. The decline generally becomes obvious at about probit 8–9. If this is so and the points in any one experiment are concentrated in the upper part of the probit range, as in the case at 6.98 g./l. and 35°C., the calculated slope will naturally be less than if the probits had been spread more evenly. Close examination of the data indicates that considerations of this kind cannot entirely account for the alterations in slope with changing concentration and temperature, but it does reveal a strong tendency towards systematic departures from the strictly linear relationship between probit and log survival-time. An analysis of the grouped data was therefore undertaken in order to establish whether or not this departure from linearity may be considered real.

The various experiments, although differing considerably in duration, all followed the same general course, and in fact they exhibit a close resemblance to one another in their results. For instance, if the points of intersection of the two parts of the probit-log survival-time graphs (assumed for this purpose

TABLE 2. The regression coefficients of probit on log₁₀ survival-time, assuming the relationship to be bilinear

Slopes of regression of probit on log ₁₀ survival-time with their standard errors								
Phenol conc. g./l.	Temp. °C.	First line		Ratio of slope to its S.E.	Second line		Ratio of slope to its S.E.	Intersection probit
		Slope	S.E.		Slope	S.E.		
7.95	20	0.6433 ± 0.0108		60	7.9063 ± 0.2737		29	4.8
	24	—	—	—	6.9940 ± 0.4513		15	—
	29	—	—	—	6.8789 ± 0.8220		8	—
	35	—	—	—	5.6174 ± 0.2177		26	—
6.98	22	—	—	—	6.6258 ± 0.6850		10	—
	25	—	—	—	6.4515 ± 0.3211		20	—
	28	—	—	—	6.8573 ± 0.5738		12	—
	30.5	—	—	—	6.3947 ± 0.5048		13	—
6.01	35	—	—	—	4.4997 ± 1.4739		3	—
	23	—	—	—	6.2644 ± 0.3666		17	—
	26	—	—	—	8.8185 ± 0.4272		21	—
	28	0.5045 ± 0.0198		26	8.7894 ± 0.5540		16	4.3
5.20	31	—	—	—	7.8776 ± 0.4020		20	—
	35	—	—	—	7.3102 ± 0.7288		10	—
	27	1.1525 ± 0.2472		4.5	11.7513 ± 0.3202		37	4.6
	31	1.2872 ± 0.1716		7.5	12.3989 ± 1.2271		10	4.6
4.62	35	—	—	—	8.5224 ± 0.7847		11	—
	39	—	—	—	6.9246 ± 0.5972		12	—
	30	—	—	—	12.2632 ± 0.6288		20	—
	30	1.2356 ± 0.0547		23	—		—	4.5
4.25	32.5	0.9184 ± 0.2291		4	11.0443 ± 0.6172		18	4.3
	35	3.5304 ± 0.2775		13	11.8884 ± 0.6143		19	4.6
	38	0.6214 ± 0.0178		35	11.0513 ± 0.5192		21	4.6
	39.5	—	—	—	7.1530 ± 0.3774		19	—
4.00	42	—	—	—	8.2696 ± 0.7842		11	—
	35	3.7462 ± 0.4695		8	13.3390 ± 0.6733		20	4.7
	35	1.6362 ± 0.1355		12	13.6191 ± 0.4417		31	4.4
	3.96	0.6501 ± 0.0907		7	11.2753 ± 0.8793		13	4.1
3.76	35	1.9524 ± 0.1235		16	12.8507 ± 0.2538		51	4.2
3.48	35	—	—	—	9.7326 ± 0.1742		56	—

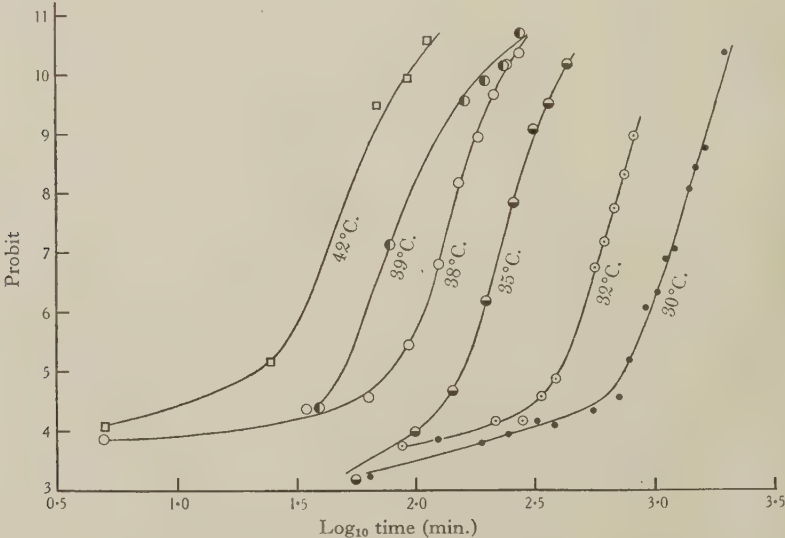


Fig. 1. Relation between probit and log₁₀ survival-time for *Bact. coli* when exposed to 4.62 g. phenol per litre at various temperatures.

to be bilinear) are calculated, it appears that the probit values at the intersection points are grouped around 4.6 (Table 2). In the cases where the data

line, is not very different from the above value. Again, the time values at the intersection points are, very roughly, a constant proportion (actually from

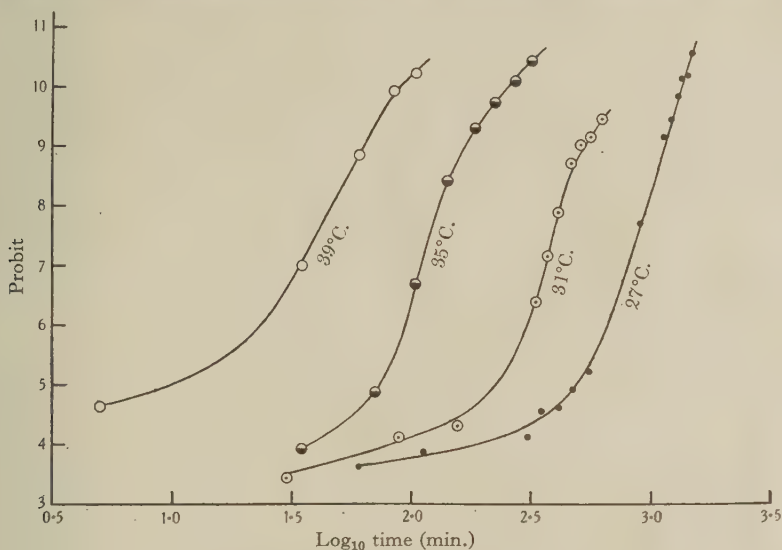


Fig. 2. Relation between probit and $\log_{10}$ survival time for *Bact. coli* when exposed to 5.20 g. phenol per litre at various temperatures.

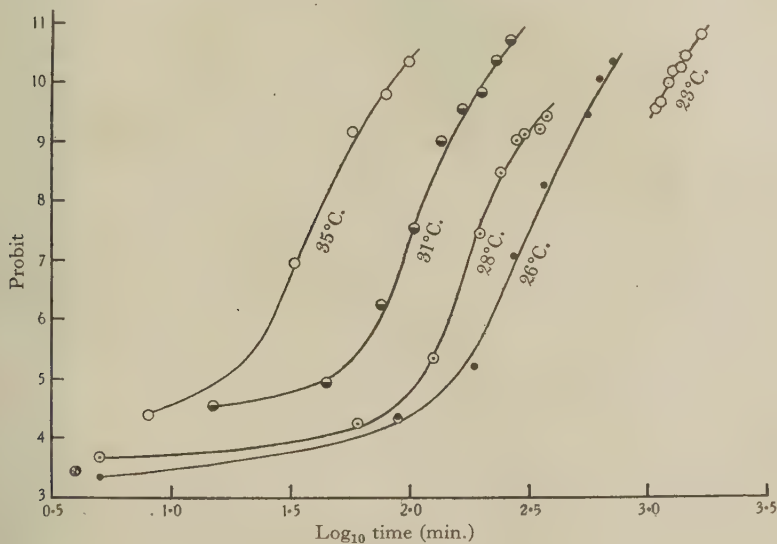


Fig. 3. Relation between probit and $\log_{10}$ survival time for *Bact. coli* when exposed to 6.01 g. phenol per litre at various temperatures.

are not sufficiently extensive for the calculation of the intersection point, the value indicated by the one or two points available, having regard to the approximate magnitude of the slope of the lower

one-third to one-fifth) of the corresponding virtual sterilization times (v.s.t.'s, where the mortality is 99.99999 %, see Jordan & Jacobs, 1944b). Finally, at corresponding stages in the different experiments,

the probit-log survival-time slopes between successive determinations are of similar magnitudes. It appeared, therefore, that the similarity between experiments was sufficiently great to allow all the data

to accomplish this have already been given (Jordan & Jacobs, 1945). The length of the expanded time scale was approximately that of the v.s.t. of the standard experiment. After thus converting the

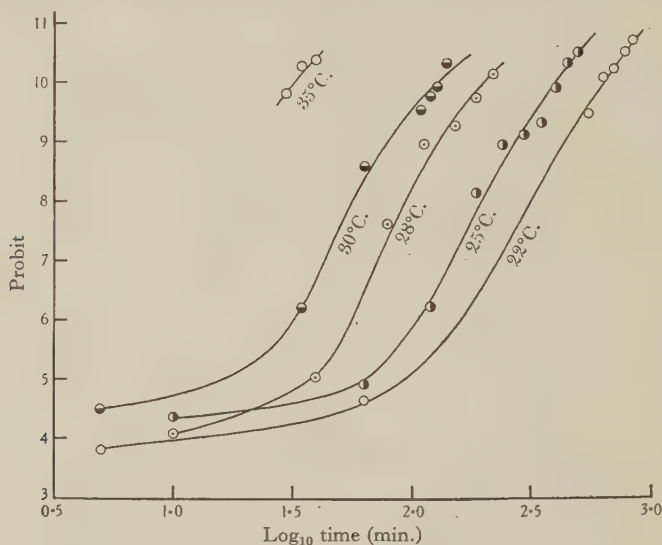


Fig. 4. Relation between probit and $\log_{10}$ survival time for *Bact. coli* when exposed to 6.98 g. phenol per litre at various temperatures.

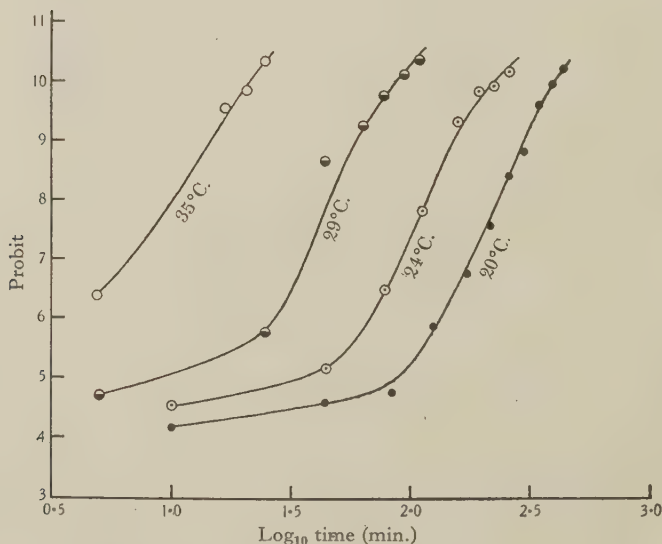


Fig. 5. Relation between probit and $\log_{10}$ survival time for *Bact. coli* exposed to 7.95 g. phenol per litre at various temperatures.

to be combined effectively. The method chosen was to expand the time scale of each experiment to the same length, i.e. that of the experiment at 4.62 g./l. and 30°C. The exact details of the method employed

times to a standard scale, a scatter diagram including all the experiments was prepared on which each successive value of the probit-log survival-time slope (Table 1) was plotted against the mid-point of the

interval of standardized time to which it corresponded.

Detailed examination of the results from the separate experiments when plotted on this scatter diagram revealed that in those experiments with long v.s.t.'s the tendency for the probit-log survival-time slope to decline towards the end of the disinfection was much less evident than in the shorter experiments. Consequently, the experiments were divided into two classes, those with v.s.t.'s greater than and those with v.s.t.'s less than 1000 min. From each class a scatter diagram was obtained and converted into a frequency table (Tables 3, 4). In the case of the

21 experiments with v.s.t.'s less than 1000 min. the grouping used was based on intervals of 200 min. of standardized time and 4 units of slope. For the data from the 7 experiments having v.s.t.'s greater than 1000 min. a coarser grouping on the standardized time scale was employed because of the fewer points available. The mean values for the slopes were obtained on the assumption that all cases in a group had slopes equal to that of the mid-point of the range for the group. From the means and frequencies the standard deviations were obtained in the usual way in order that the scatter of the values around the mean might be estimated. It appears that when the

TABLE 3. *Frequency diagram of slopes of probit-log time lines for all experiments with v.s.t.'s less than 1000 min.*

		Standardized time scale (min.)											
		0	200	400	600	800	1000	1200	1400	1600	1800	2000	2400
Slope of probit-log time line	24	—	—	—	—	—	—	—	—	—	—	—	—
	20	—	—	—	—	—	2	2	1	—	—	—	—
	16	—	—	—	1	1	4	1	1	—	1	—	—
	12	—	—	3	8	7	5	2	1	6	4	—	—
	8	—	—	8	3	4	—	9	2	9	11	3	—
	4	8	12	4	—	—	1	4	4	3	3	1	—
Total	0	8	12	15	12	12	12	18	9	18	19	4	—
Mean		2.0	2.0	5.7	9.3	9.0	12.0	7.3	6.9	6.7	6.6	5.0	—
Standard deviation		—	—	2.82	2.31	2.49	4.35	4.40	5.92	2.83	3.06	2.00	—

TABLE 4. *Frequency diagram of slopes of probit-log time lines for all experiments with v.s.t.'s exceeding 1000 min.*

		Standardized time scale (min.)					
		0	400	800	1200	1700	2200
Slope of probit-log time line	24	—	—	—	—	—	1
	20	—	—	—	3	—	1
	16	—	—	4	6	—	5
	12	—	—	5	5	—	2
	8	—	6	3	—	—	2
	4	8	5	—	1	—	2
Total	0	8	11	12	15	—	13
Mean		2.0	4.2	10.3	12.7	—	11.2
Standard deviation		—	2.09	3.17	4.19	—	5.97

experiments are combined in the manner described there is clear evidence for the existence of a well-marked maximum in the slope of the probit-log survival-time line. This occurs in the combined data when about half the v.s.t. has elapsed, but in the individual experiments it may occur relatively earlier when their duration is short. Tables 3 and 4 and Fig. 6 illustrate well the initial period of small slope, and the transition between this and the following stage is fairly abrupt. After the slope has reached its maximum value in the class of experiments with v.s.t.'s less than 1000 min. there is a sharp decline to an almost constant level with, nevertheless, a tendency towards a continuous fall (Fig. 6A). In the experiments having v.s.t.'s greater than 1000 min. the slope of the probit-log survival-time line rises fairly sharply from the initial low level to a maximum at which it tends to remain constant (Fig. 6B). The interrupted lines in the figure represent the means plus and minus half their standard deviations in order to afford visual presentation of the scatter of the individual values around the means.

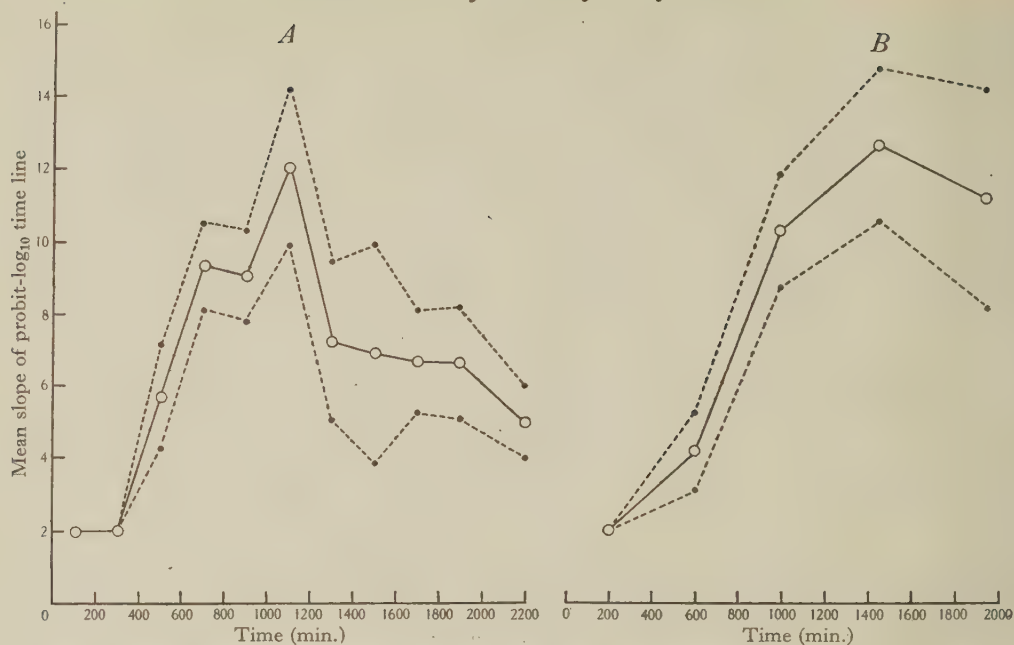


Fig. 6. Relation between mean slope of probit-log₁₀ time line and standardized survival time (see text).
A = All experiments with v.s.t.'s less than 1000 min. *B* = All experiments with v.s.t.'s greater than 1000 min.

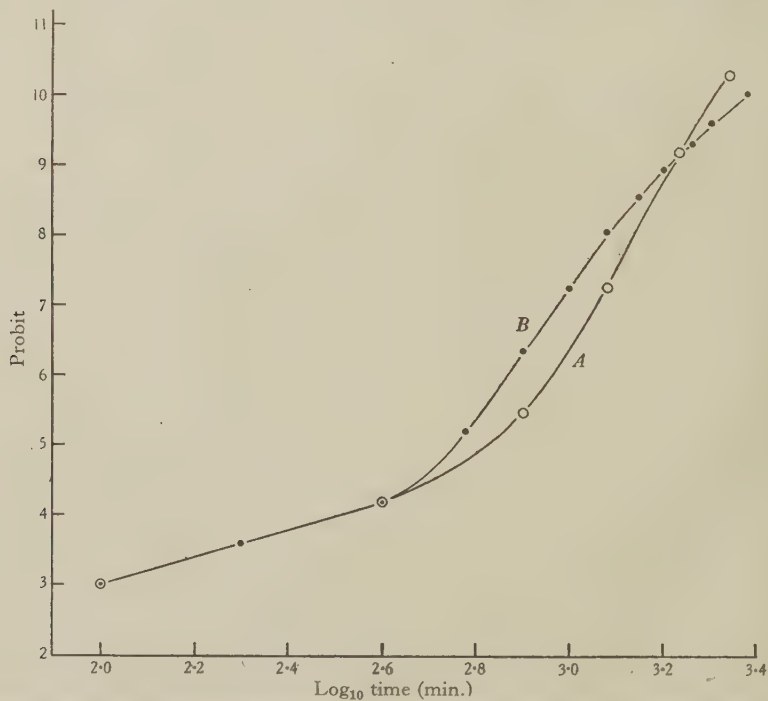


Fig. 7. Idealized probit-log₁₀ survival-time curves constructed from mean data in Tables 3 and 4 and in Fig. 6.

It is a simple matter to convert the data in Tables 3 and 4 back into 'idealized' probit-log survival-time graphs. The results of this reversion are shown in Fig. 7, where curve *A* represents the class of longer and curve *B* the class of shorter experiments. In both cases the point of origin has been taken as probit 3 at 100 min. These curves clearly epitomize the different types of data obtained experimentally (cf. Figs. 1-5). The curvature of the portion of curve *B* (Fig. 7) above probit 5 is very evident, but this is partly due to the large number of points used to determine this portion of the curve. With fewer points there would have been greater reason to draw the graph as a straight line. Indeed, if curve *B* above probit 5 is treated as if it were linear, the calculated slope is found to be 8.15 ± 0.325 , the standard error being only one twenty-fifth of the slope, despite the fact that this part of curve *B* was constructed from data in which the slope varied between 12 and 5. It should be emphasized that through the use of very large numbers of organisms probit values above 8 have been secured, and it is in this region that the change in slope becomes apparent. There would seem to be no good reason why curve *A*, although it approximates very closely to the linear form with a slope of 11.47 ± 0.347 , should not also really be of the same general form as curve *B*. It may be that if sufficient experiments, each with many determinations of survivors, had been available, there would have been revealed a gradual decrease in the curvature of the part above probit 5 as the v.s.t. lengthened.

If, then, it is accepted that the upper part of the probit-log survival-time graph is really a curve, it would seem logical to suppose that the lower part is also curved although possibly only slightly, and that there is a smooth though rapid transition between the two parts. The bilinear treatment of the graphs was unsatisfactory in that it was difficult to account for the sudden change in slope. If, however, it is accepted that all these graphs are fundamentally very

asymmetrical sigmoid curves, then this difficulty disappears. In fact, since the probit-time curves for all these experiments are sigmoid (Jordan & Jacobs, 1944*a*), and as the substitution of log time for time leads to a relative expansion of the early part and a contraction of the later part of a disinfection, the probit-log survival-time graphs would naturally tend to assume the very asymmetrical sigmoid shape of curve *B* (Fig. 7) which in turn approximates to the bilinear form. Bliss (1935) refers to the frequent appearance of bilinear probit graphs in dosage-mortality data. It is possible that these are, in reality, cases of very asymmetrical sigmoid curves of the type under consideration here, the true nature of which would have been revealed had the numbers of organisms used for the tests been sufficient to yield the necessary high probit values. As stated above, it is the probit values above 8 that, in the present instance, gave the clue to the true nature of the probit-log survival-time curves.

It remains to be considered to what extent the probit-log survival-time graphs can legitimately be used for the calculation of mortality times. It would appear that when the disinfection process is slow the graphs approximate very closely to the bilinear form and the calculated straight regression lines should give good values for any desired degree of mortality, except for the uncertainty around the point of intersection. This, unfortunately, lies very near to probit 5, corresponding to the important 50% mortality. In the case of shorter experiments the graphs could only be treated as linear over short ranges and extrapolation of such lines would not be permissible. This is evident from curve *B*, Fig. 7. Values for the v.s.t. could, however, be derived from the experimental points above about probit 8.5-9, since here the slope of the graph changes only very slowly. But this procedure would appear to offer no advantage over the method previously advocated for calculating the v.s.t. (Jordan & Jacobs, 1945) based on the final stage of the log survivors-time curve.

REFERENCES

- BLISS, C. I. (1935). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.
 CHICK, H. (1930). *A System of Bacteriology in Relation to Medicine*, **1**, 179. London: H.M. Stationery Office.
 JORDAN, R. C. & JACOBS, S. E. (1944*a*). Studies in the dynamics of disinfection. I. New data on the reaction between phenol and *Bact. coli* using an improved technique, together with an analysis of the distribution of resistance amongst the cells of the bacterial populations studied. *J. Hyg., Camb.*, **43**, 275.
 — (1944*b*). Studies in the dynamics of disinfection. II. The calculation of the concentration exponent for phenol at 35°C. with *Bact. coli* as test organism. *J. Hyg., Camb.*, **43**, 363.
 JORDAN, R. C. & JACOBS, S. E. (1945). Studies in the dynamics of disinfection. III. The reaction between phenol and *Bact. coli*: The effect of temperature and concentration, with a detailed analysis of the reaction velocity. *J. Hyg., Camb.* (in the Press).
 RAHN, O. (1932). *The Physiology of Bacteria*. Philadelphia: Blakiston.
 — (1943). The problem of the logarithmic order of death in bacteria. A critical discussion. *Biodynamica*, **4**, 81.
 WITHELL, E. R. (1942). The significance of the variation in shape of time-survivor curves. *J. Hyg., Camb.*, **42**, 124.

(Received 17 November 1944)

The use of abrasives in the transmission of plant viruses

BY H. KALMUS, *Department of Biometry, University College, London*

AND B. KASSANIS, *Rothamsted Experimental Station, Harpenden, Herts*

(With Plate 8)

The effect of different abrasives on the transmissibility of several plant viruses was tested. Celite and animal charcoal were as effective as carborundum in increasing the number of lesions produced by a given inoculum: 400-mesh carborundum was the most effective among the different sizes which were tested; this gave a result equivalent to increasing the virus content a hundred times. Some preparations of carborundum and charcoal reduced infectivity.

Uninjured plants resisted infectivity when virus solutions were sprayed over them. Leaves previously rubbed without abrasives developed only few lesions whereas leaves rubbed with abrasives developed large numbers. Three hours after rubbing with abrasives leaves had regained their resistance to sprayed virus solutions.

The effects of rubbing leaves with abrasives are described and their significance discussed.

INTRODUCTION

Some viruses which are difficult to transmit by inoculation are readily transmitted if a suitable abrasive is incorporated in the inoculum. The first abrasive used was sand (Fajardo, 1930; Samuel & Bald, 1933), but the more effective carborundum is now generally used (Rawlins & Tompkins, 1934, 1936; Bawden, 1936; Black, 1938). How carborundum facilitates transmission is still uncertain, but cytological studies of Rawlins & Tompkins (1936) showed that the carborundum particles pierce the epidermis without killing the parenchymatous cells beneath.

The effects of carborundum are not restricted to viruses normally difficult to transmit, for it also increases the number of local lesions produced by such viruses as tobacco mosaic and severe etch. Such viruses have obvious advantages for studying the phenomenon, for they allow quantitative experiments. Using them, Costa (1944) was able to find that 500-mesh carborundum was the optimum size for increasing transmission. Other substances, less obviously abrasive, also increase infectivity. Stanley (1935) and Bald (1937) found some increase in numbers of lesions when charcoal was added to tobacco mosaic virus. Bald suggested that the effect was due to charcoal acting as a spreader and causing more even distribution of the inoculum over the rubbed leaves. Costa (1944) found that aluminium oxide also considerably increases the infectivity.

The present paper describes comparative experiments with these and other substances and gives some information that may help in interpreting their mode of action. The viruses used were tobacco mosaic, bushy-stunt, potato *X* and the Rothamsted culture (Bawden & Pirie, 1942) of tobacco necrosis. The first two were used as purified preparations and the last as infective sap clarified by centrifuging for

10 min. at 10,000 r.p.m. Local lesion tests were made with tobacco mosaic and bushy-stunt viruses on *Nicotiana glutinosa*, with potato virus *X* on tobacco, var. White Burley, and with tobacco necrosis virus on *Phaseolus vulgaris* var. Canadian Wonder. The tests were made either by the half-leaf method, or Latin square or some other randomized design to reduce error arising from variations in susceptibility of individual leaves. It was found that the best method of applying the substances under test was to shake them over the leaves with a small paint brush. The leaves were then rubbed over their whole surface as uniformly as possible with the forefinger wet with inoculum. If virus preparations were used at the dilutions usual in local lesion tests, so many lesions were obtained with some of the substances used that the inoculated leaves collapsed and no accurate count could be made. It was therefore necessary to use the viruses at such dilutions that very few lesions were produced by the control leaves.

COMPARISON OF DIFFERENT ABRASIVES

Table 1 gives the results of one of our earliest tests comparing the relative efficiencies of different substances in increasing the lesions produced by three viruses. It shows that a variety of substances can increase the number of lesions produced by the three viruses, but it is included mainly because of the unexpected result obtained with carborundum. This was a commercial sample of unknown origin compacted into cake-form, but easily turned into a normal-looking powder by light scraping. It has been tested many times with different viruses and has always reduced the number of lesions. It seems probable that its inactivating effect is due to its high

alkalinity, since water-suspensions containing it have a pH value around 13.

Table 2 shows that its behaviour is not typical of carborundum, as five new samples from the Manchester Carborundum Co. all increased the infectivity of tobacco mosaic virus, four of them being more effective than animal charcoal which was the best of the materials listed in Table 1. Before samples of commercial carborundum are used to facilitate the transmission of viruses not otherwise

that most samples give only moderate increases in the number of local lesions, whereas animal charcoal gives large increases and one sample of carbon reduced the number. The last sample, probably lamp-black, was oily and constituents other than carbon may have been responsible for the loss of infectivity. Differences in the behaviour of the other preparations may also be due to the presence of other materials or to differences in the size of the carbon particles.

TABLE 1. *Effect of different abrasive substances on the infectivity of three virus preparations**

Substances	Viruses and hosts		
	Tobacco mosaic <i>N. glutinosa</i>	Tomato bushy-stunt <i>N. glutinosa</i>	Tobacco necrosis <i>Ph. vulgaris</i>
Control	100	100	100
Celite (diatomaceous silica) product of Johns-Manvill	509	1022	1206
Animal charcoal (purified) product of Hopkin and Williams	634	1350	1000
Plant charcoal product of Merck	568	665	338
Al ₂ O ₃ calcinized product of the British Drug Houses Ltd.	324	945	778
Kaolin	128	458	—
Compacted carborundum of unknown origin	54	—	47

* Purified tobacco mosaic and tomato bushy-stunt viruses were used at a concentration 10^{-8} g./ml. and tobacco necrosis virus as clarified sap diluted 1/50. The numbers of lesions are presented as percentage of the control. The average number of lesions per leaf for the control was 59 with tobacco mosaic, 18 with bushy-stunt and 12 with tobacco necrosis virus.

TABLE 2. *Effect of size of carborundum particles on the infectivity of tobacco mosaic virus**

Size and type of abrasive	Relative no. of lesions on <i>N. glutinosa</i>
Without abrasive	100
Carborundum 100 mesh	830
Carborundum 180 mesh	1700
Carborundum 220 mesh	3100
Carborundum 400 mesh	5630
Carborundum 600 mesh	2330
Animal charcoal	1430

* Tobacco mosaic virus was used at a concentration 10^{-8} g./ml. The numbers of lesions are presented as percentage of the control. The average number of lesions per leaf for the control was 0.4.

easily transmitted, it is clear that they should be tested with some such virus as tobacco mosaic to ensure that they are effective. It is also clear from Table 2 that the particle size of the abrasive is of considerable importance. Our results agree with those of Costa (1944), who found that 500 mesh was the most effective of the grades he tested.

The increases in infectivity described by Stanley (1935) and Bald (1937) when using charcoal in their inocula were small compared with those shown in Tables 1 and 2. As with carborundum, we have found that different samples of charcoal also vary widely in their behaviour (Table 3). It will be seen

TABLE 3. *Effect of different preparations of charcoals on the infectivity of tobacco mosaic virus**

Preparation	Relative no. of lesions on <i>N. glutinosa</i>	Description
Control	100	—
Animal charcoal	2120	Clumps and sticks to leaves after washing, feels coarser than plant charcoal
Plant charcoal	390	Clumps and sticks to leaves even more than animal charcoal
Bone charcoal	330	Does not stick to leaves, feels coarser than animal charcoal
Commercial charcoal:		
Sample A	300	Similar to bone charcoal
Sample B	0	Oily, very fine, sticks persistently to leaves

* Tobacco mosaic virus was used at a concentration 10^{-7} g./ml. The numbers of lesions are presented as percentage of the control. The average number of lesions per leaf of the control was 2.3.

Costa (1944) has shown that shaking carborundum with tobacco mosaic virus and then removing the carborundum by filtering through filter paper has no effect on the virus. He concludes from this that

the effect is in the plant. Animal charcoal, however, has much the same effect on transmission as carborundum, yet this behaves very differently in such tests. Table 4 shows the infectivity of filtrates from mixtures of tobacco mosaic virus with various materials. It is clear that no deduction can be made about the action of these materials from such an experiment, for some materials that are effective in increasing infection adsorb the virus whereas others do not.

The effect of adding abrasives to inoculum is clearly similar to increasing the concentration of virus. We have attempted to measure the effect by making local lesion tests with virus solutions at different concentrations with and without abrasive.

TABLE 4. Ability of different abrasives to adsorb tobacco mosaic virus*

Type of abrasive	Av. no. of lesions per leaf on <i>N. glutinosa</i>	pH of the suspension
Control	10	7
Carborundum 400 mesh	8	
Carborundum 100 mesh	11	
Animal charcoal	0	5.4
Plant charcoal	0	4.0
Celite	11	5.9

* 10% suspensions of the abrasives were made in solutions containing 10^{-6} g./ml. of tobacco mosaic virus. The fluids were filtered through filter paper and the filtrates tested for their infectivity.

TABLE 5. Effect of carborundum 400 mesh on the dilution end-point of tobacco mosaic virus

Treatments	Av. no. of lesions per leaf at different virus concentrations (g./ml.)					
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Leaves dusted with carborundum	407	166	26	5	1	0
Control leaves	24	4	2	0	0	0

One such test with carborundum 400 mesh is shown in Table 5, and it will be seen that the addition of carborundum was equivalent to increasing the virus concentration 100 times. The number of lesions produced by tobacco mosaic virus solutions containing celite or charcoal is usually equal to the number given by ordinary inoculum at about 50 times the concentration and the dilution end-point is increased by from 50 to 100 times. With other viruses and hosts, similar increases are obtained.

In *Nicotiana glutinosa* plants the upper hairy leaves usually give many fewer lesions with tobacco mosaic virus than do the lower leaves. It is on these upper leaves that the incorporation of an abrasive has the most pronounced effect. When opposite half-leaves of the plant are rubbed with the same inoculum with and without an abrasive, the increase in the number

of lesions due to the abrasive is 5-10 times greater on the upper than on the lower leaves. Of the various materials that we have used for increasing ease of transmission, we prefer celite. This is a little less effective than 400 mesh carborundum or animal charcoal, but it has other advantages. When using carborundum on fragile plants, it is difficult to avoid causing some damage. Charcoal also leads to damage, but its main disadvantage is that it sticks to the leaf and is not readily washed off. These disadvantages are probably not of great importance when systemic infection is needed, but they are if materials are needed to incorporate into quantitative local lesion tests. The relative efficiencies of the different materials that we have tested seem to be the same regardless of the virus. In addition to the viruses already mentioned, we have used potato viruses X and Y, severe etch and tobacco ringspot viruses.

INFECTION OF PLANTS BY SPRAYING

Most workers are agreed that viruses only enter plants through wounds, but a few have obtained infection by spraying what they believed to be uninjured plants and attributed this to infection through stomata. The work on this subject has been reviewed by Price (1938), who has also made detailed tests from which he concluded that occasional infection obtained by spraying plants with virus suspensions resulted from wounds produced by the rubbing together of leaves. Experiments we have made confirm this conclusion. Plants kept in conditions in which their leaves could not be damaged by contact with one another have never produced any lesions when suspensions of tobacco mosaic, bushy-stunt, potato virus X and tobacco necrosis viruses have been sprayed over them with an atomizer. When leaves have been injured, however, lesions are produced by such spraying.

When infections are made by spraying plants with virus suspensions the effect of abrasives in increasing susceptibility is even more pronounced than when infections are made by rubbing inoculum over the leaves (Pl. 8, Fig. 1). It will be seen from Table 6 that no lesions were produced in undamaged halves of *N. glutinosa* leaves by spraying with suspensions of tobacco mosaic or bushy-stunt viruses, only very few when the half-leaves were previously rubbed only with water, whereas many hundreds of lesions were produced on opposite half-leaves previously rubbed with celite. The effect of rubbing in producing entry point is temporary and, 3 hr. after the rubbing, even the leaves treated with celite have again become resistant to infection by spraying. The temporary nature of the entry points produced by wounding may explain the fact that fewer lesions are produced when the virus is sprayed over a previously rubbed leaf than when the inoculum is itself rubbed

over the leaf. This is so whether abrasive is used or not (Table 6, treatments 6 and 7).

Treatment 5 in Table 6 shows that spraying leaves previously rubbed with celite actually produces more lesions than when the inoculum is rubbed directly over the leaves without abrasive. This effect is also given by carborundum and animal charcoal. An experiment with animal charcoal and tobacco mosaic

lesion tests equivalent to increasing the virus content by 100 times. It is probable that the viruses normally difficult to transmit by the inoculation of infective sap, but which are readily transmitted by the use of abrasive, are those that occur in infective sap at or below the dilution end-point required for their respective hosts. It is significant that all these viruses have been occasionally transmitted without the use

TABLE 6. *Effect of previous treatment on the susceptibility of N. glutinosa to infection when sprayed with virus preparation**

Experiments	1st treatment on opposite half-leaves	2nd treatment on whole leaves	No. of lesions	
			Bushy-stunt virus	Tobacco mosaic virus
1	Not treated	Both halves sprayed and washed immediately after 1st treatment	0	0
	Dusted with celite, rubbed with water		492	401
2	Rubbed with water	As Exp. 1	4	23
	Dusted with celite, rubbed with water		452	468
3	As Exp. 2	Sprayed and washed 1 hr. after 1st treatment	7	1
			71	37
4	As Exp. 2	Sprayed and washed 2 hr. after 1st treatment	0	—
			3	—
5	Rubbed with inoculum	As Exp. 1	184	224
	Dusted with celite and rubbed with water		428	442
6	Rubbed with inoculum	As Exp. 1	178	252
	Rubbed with water		6	15
7	Dusted with celite and rubbed with water	As Exp. 1	—	410
	Dusted with celite and rubbed with inoculum		—	1240

* Tobacco mosaic virus was used at a concentration of 10^{-5} and bushy-stunt virus at 10^{-7} g./ml. The numbers of lesions in each experiment are totals from 7 opposite half-leaves.

virus at different concentrations is shown in Table 7. Opposite halves of *N. glutinosa* leaves were treated in different ways: one was rubbed with the forefinger dipped in inoculum and the other dusted with charcoal and rubbed with water. The leaves were then sprayed with the same concentration of tobacco mosaic virus. It will be seen that over the whole range of dilutions more lesions were produced on the half leaves treated with charcoal.

DISCUSSION

Although the dilution end-point of infective sap is widely used to characterize viruses, it has no constant value and cannot be considered an intrinsic property. Indeed, with a given sample of sap it will vary with the method of inoculation and the host plant inoculated. Sap from tobacco plants infected with potato virus Y, for example, gives a dilution end-point of 1/10,000 when inoculated to tobacco plants and an end-point of about 1/500 when inoculated to President potatoes. The results described in this paper show that the incorporation of suitable abrasives in the inoculum may increase the dilution end-point of a given virus preparation by 100 times and when virus concentrations are small gives an effect in local

TABLE 7. *Relative efficiency of spraying after treatment with abrasive and inoculating without abrasive*

Treatment	Av. no. of lesions per half-leaf of <i>N. glutinosa</i> at different concentrations (g./ml.)			
	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Half-leaves rubbed with animal charcoal and then sprayed with inoculum	426	297	111	41
Opposite half-leaves rubbed with inoculum, without charcoal	137	32	7	3

of abrasive, whereas abrasives do not help with the transmission of curly-top virus which is believed not to be transmitted mechanically because it needs to be placed directly into the phloem (Bennett, 1934). Results with potato virus A (Bawden, 1936) support the idea that low virus content of infective sap rather than any intrinsic property of the virus is responsible for failure of transmission. This virus is transmitted from potato to tobacco without any abrasive, but not from potato to potato. The dilution end-point when tested to tobacco is about 1/50. Clearly, if potato and tobacco have the same relative susceptibility to

virus *A* as to virus *Y*, the failure to infect potato is simply explained by the virus content being below the dilution end-point for potato. Successful transmission to potato when carborundum is incorporated in the inoculum merely reflects the ability of the abrasive to increase the dilution end-point beyond that required for infection of the potato.

The apparent effect of abrasives is similar to increasing the virus content, but clearly as it cannot do this it must be acting by reducing the resistance of the host. One possible explanation for this is that abrasives merely increase the number of wounds produced by rubbing and so increase the likelihood of virus particles entering the plants.

The enormous effect abrasives have on the rubbed leaf is clearly shown by ammoniacal silver hydroxide treatment (argentaffin test). In the presence of light, this reagent turns black when it reacts with any reducing substance. When unwounded leaves (*N. glutinosa*, tobacco, potato, sugar beet, lettuce) are placed in the reagent they show no change. When leaves rubbed with the forefinger are treated, they become stippled with black spots. With hairy leaves the spots are produced mainly at the bases of broken trichomes. When leaves rubbed with abrasive are treated, these become evenly blackened (Pl. 8, fig. 2). This increase in the permeability of the leaf is probably due to the complete removal of the outer waxy layer by the abrasive, which may play a part in influencing the entry of viruses. However, penetration of virus and of silver hydroxide are not necessarily correlated, for the results in Table 6 showed that leaves rubbed

with abrasive recovered their resistance to infection by sprayed virus within 3 hr., whereas it was found that they continue to give the argentaffin test days after rubbing. It seems that the injured leaves quickly repair damage to their semi-permeable systems that prevent the entry of large particles, but cannot repair damage to the cuticular layer that prevents entry of salts.

A second possibility is that abrasives facilitate transmission because they affect different cells from those affected by ordinary rubbing and that the two types of cells differ in their susceptibility to infection. With ordinary rubbing the maximum number of lesions that a leaf can carry will be produced if the inoculum is sufficiently concentrated, so that there is no actual shortage of entry points. It may be that with dilute inocula there are insufficient individual virus particles to meet all these entry points, but it is at least as likely that these entry points are into a type of cell that succumbs only to mass inoculum.

In addition to removing the cuticular layer, rubbing with abrasive also strips off all the trichomes. This may expose cells not affected by ordinary rubbing, for the argentaffin test shows that it is mainly bases of trichomes that are affected by rubbing without abrasives. It is possible that trichomes are more resistant to infection—that is, more initial virus particles are needed to give an infection—than are the epidermal cells of the leaf. If this be so, then the effects of abrasives may be to open these more susceptible cells which are normally undamaged by rubbing.

REFERENCES

- BALD, J. G. (1937). The effect of carbon on the production of lesions by viruses of the tobacco mosaic group. *Ann. appl. Biol.* **24**, 77.
- BAWDEN, F. C. (1936). The viruses causing a top necrosis (acronecrosis) of the potato. *Ann. appl. Biol.* **23**, 487.
- BAWDEN, F. C. & PIRIE, N. W. (1942). A preliminary description of preparations of some of the viruses causing tobacco necrosis. *Brit. J. exp. Path.* **23**, 314.
- BENNETT, C. W. (1934). Plant tissue relations of the sugar-beet curly-top virus. *J. agric. Res.* **48**, 665.
- BLACK, L. M. (1938). Properties of the potato yellow-dwarf virus. *Phytopathology*, **28**, 863.
- COSTA, A. S. (1944). Quantitative studies with carborundum and its use in local-lesion tests. *Phytopathology*, **34**, 288.
- FAJARDO, T. G. (1930). Studies on the mosaic disease of the bean (*Phaseolus vulgaris* L.). *Phytopathology*, **20**, 469.
- PRICE, W. C. (1938). Studies on the virus of tobacco necrosis. *Amer. J. Bot.* **25**, 603.
- RAWLINS, T. E. & TOMPKINS, C. M. (1934). The use of carborundum as an abrasive in plant-virus inoculation. *Phytopathology*, **24**, 1147.
- (1936). Studies on the effect of carborundum as an abrasive in plant-virus inoculation. *Phytopathology*, **26**, 578.
- SAMUEL, G. & BALD, J. G. (1933). On the use of the primary lesions in quantitative work with two plant viruses. *Ann. appl. Biol.* **20**, 70.
- STANLEY, W. M. (1935). Some effects of different chemical agents on infectivity. *Phytopathology*, **25**, 899.

EXPLANATION OF PLATE 8

Fig. 1. Infection by spraying *N. glutinosa* leaves with a dilute solution of tobacco mosaic virus. Left-hand half of leaf previously rubbed with celite, right-hand half previously rubbed with water.

Fig. 2. Effect of rubbing *N. glutinosa* leaves with celite on their permeability to ammoniacal silver hydroxide (argentaffin test). Left-hand half rubbed with water, right-hand half rubbed with celite.

(Photographs by V. Stansfield, F.R.P.S.)

(Received 26 January 1945)



Fig. 1.

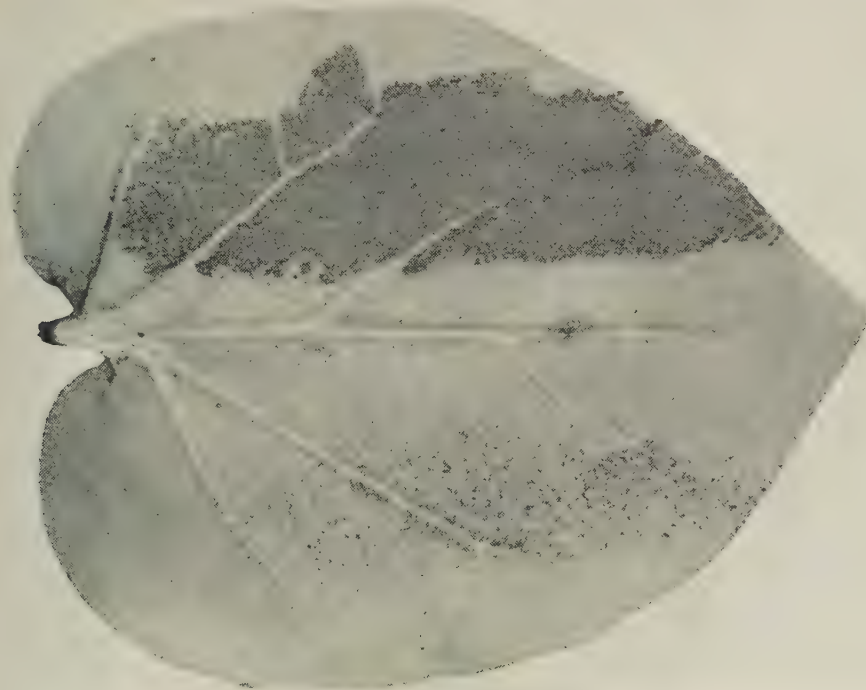


Fig. 2.

Changes in wireworm population associated with cropping

By H. W. MILES AND M. MILES, *Research Station, Long Ashton, Bristol*

Data are given to show that the rate of decline of the wireworm population associated with a root crop is not significantly greater than that associated with a cereal crop. Data are given on the wireworm populations of adjoining ley plots over 3-5 successive years, and indicate a slow rate of increase during the early years.

INTRODUCTION

Investigations on changes in wireworm population associated with cultivation and cropping were begun at Warburton, Cheshire (Field Station, Agricultural Advisory Department, University of Manchester), in 1937, and preliminary observations have been published (Miles, 1942). It is general experience that the breaking of grass land and its subsequent cropping leads to a reduction in the wireworm population (Anon., 1944), and it has been generally accepted that crops receiving much cultivation are associated with greater reductions in the wireworm population than those receiving little cultivation. Root crops are considered to create conditions especially unfavourable to the survival of wireworms, since hoeing and cultivating are done at a time when wireworms are active near the surface, and the extensive proportion of soil left bare until after midsummer makes conditions unfavourable for egg-laying by click beetles (Gough & Evans, 1942) and the survival of newly hatched wireworms. Studies of fluctuations in wireworm populations as a result of cropping and associated cultural operations have been limited by technical difficulties, and the effects of major cultural operations such as ploughing and disking have not been distinguished from those of harrowing and hoeing that affect only the surface soil. In this paper the writers' observations on the rates of decline in wireworm population associated with potato crops having a maximum of cultivation and cereals which had a minimum of cultivation are described.

Sampling method. Estimations of wireworm populations were based on hand-sorting wireworms from 8 in. cubes of soil. The efficiency of the method has already been discussed (Miles, 1942), and comparisons by Hollick who used the Salt & Hollick extraction apparatus (Salt & Hollick, 1944) indicated that in the light, sandy soil of Warburton (Stewart, 1938) few wireworms exceeding 5 mm. long were missed. The plots under observation were 25 x 10 yd., and each season after cropping, between October and March, fifty 8 in. cubes of soil were examined from each plot. The wireworm population estimates given in Tables 1-3 are based, therefore, on the examination of practically 1% of the area of the plots.

Wireworms of *Agriotes* spp. were predominant at Warburton, but other species also occurred. In the winter of 1941-2 the proportions of the various species recorded were: *Agriotes* Esch., 79%; *Athous haemorrhoidalis* F., 1%; *Cryptohypnus riparius* F., 9%; *Adrastus limbatus* F., 11%.

FALL IN WIREWORM POPULATION ASSOCIATED WITH POTATO CROPS

Plots intended for potatoes were dug during winter and early spring. Later they were ridged, manure (farmyard and compost) was put in the trenches along with the potato sets, and the ridges were then split to cover the potatoes. Cultivating and ridging kept the ground free from weeds, and the plants did not meet across the furrows until after midsummer. Table 1 indicates wireworm populations per acre before and after cropping with potatoes during the period 1938-42.

TABLE 1. *Wireworm populations per acre before and after cropping with potatoes*

Before	After
199,000 ± 27,000	98,000 ± 14,000
317,000 ± 27,000	145,000 ± 21,000
108,000 ± 21,000	80,000 ± 12,000
111,000 ± 18,000	245,000 ± 33,000
108,000 ± 21,000	94,000 ± 19,000
372,000 ± 43,000	69,000 ± 14,000
80,000 ± 14,000	67,000 ± 14,000
235,000 ± 29,000	147,000 ± 39,000
343,000 ± 30,000	63,000 ± 15,000
186,000 ± 28,000	53,000 ± 10,000
356,000 ± 40,000	265,000 ± 43,000
176,000 ± 41,000	90,000 ± 13,000
147,000 ± 20,000	98,000 ± 21,000
186,000 ± 35,000	76,000 ± 14,000
114,000 ± 16,000	151,000 ± 24,000
67,000 ± 11,000	51,000 ± 13,000
257,000 ± 32,000	88,000 ± 17,000
* 102,000	69,000 ± 13,000
* 76,000	82,000 ± 14,000
* 26,000	63,000 ± 10,000
* 78,000	22,000 ± 6,000
* 114,000	59,000 ± 9,000
* 37,000	47,000 ± 10,000
25,000 ± 7,000	8,000 ± 3,000
49,000 ± 10,000	10,000 ± 4,000
39,000 ± 8,000	4,000 ± 3,000
20,000 ± 7,000	4,000 ± 3,000
Mean population:	
145,000 ± 23,000	83,000 ± 15,000

* Counts for individual samples not available.

FALL IN WIREWORM POPULATION ASSOCIATED WITH SPRING CEREAL CROPS

In the rotation on the Warburton plots spring cereals followed roots and potatoes. Roots and potatoes were lifted with a fork in the autumn and the plots were harrowed during the winter to keep them free from

weeds. Oats and wheat were drilled in spring and the plots were rolled after the seedlings began to show. Table 2 gives wireworm populations before and after spring cereal crops grown 1938-42: since oats and wheat had similar cultivations, they are not considered separately.

TABLE 2. Wireworm populations per acre before and after cropping with spring cereal crops

Before	After
53,000 ± 10,000	10,000 ± 5,000
265,000 ± 43,000	63,000 ± 12,000
90,000 ± 13,000	22,000 ± 8,000
98,000 ± 21,000	43,000 ± 9,000
76,000 ± 14,000	43,000 ± 9,000
35,000 ± 9,000	* 76,000
88,000 ± 17,000	* 114,000
69,000 ± 13,000	* 37,000
82,000 ± 14,000	18,000 ± 6,000
74,000 ± 15,000	43,000 ± 11,000
63,000 ± 10,000	24,000 ± 6,000
22,000 ± 6,000	25,000 ± 7,000
59,000 ± 9,000	31,000 ± 8,000
47,000 ± 10,000	39,000 ± 10,000
98,000 ± 14,000	20,000 ± 6,000
145,000 ± 21,000	63,000 ± 11,000
94,000 ± 19,000	45,000 ± 13,000
69,000 ± 14,000	169,000 ± 24,000
67,000 ± 14,000	61,000 ± 12,000
147,000 ± 39,000	110,000 ± 22,000
49,000 ± 10,000	* 55,000
12,000 ± 6,000	10,000 ± 4,000
43,000 ± 10,000	47,000 ± 9,000
113,000 ± 24,000	57,000 ± 11,000
* 174,000	112,000 ± 14,000
Mean population:	
85,000 ± 16,000	53,000 ± 10,000

* Counts for individual samples, not available.

DISCUSSION

Data given in Tables 1 and 2 indicate that the mean rate of fall in the wireworm population on twenty-seven plots of potatoes was 43 % and on twenty-five plots of spring cereals was 38 %. The difference, 5 %, is not significant, though it may indicate some extra loss of wireworms on potato plots through

mechanical injury during digging and trenching. It seems likely that cultivation may have less effect on wireworm populations than has been thought, particularly those cultivations that affect mainly the surface soil. Recent work of Lees (1943) has emphasized the sensitivity of wireworms to humidity and moisture conditions and has shown that wireworms migrate readily from an unfavourable environment. Wireworms are not generally found in the layer of dry surface soil, bare of cover and without plant roots, that occurs during the early stages of growth of root crops. They are found in the moister soil below and tend to be concentrated among the crop roots: in consequence, they are not much disturbed by surface cultivations that aim at the removal of weeds and the conservation of moisture. Undoubtedly major operations such as digging, ploughing and disking cause mechanical injury to many wireworms and expose numbers to the immediate attacks of birds, but those wireworms able to do so speedily leave exposed soil at the onset of desiccation, and by the time the surface soil is reduced to a tilth, either by weathering or by further cultivation, it seems unlikely that any considerable proportion of the wireworms remain in it.

Increase in wireworm population under ley

Table 3 gives data on the increase in the number of wireworms when the land is undisturbed under ley.

Table 3 shows that the wireworm population gradually increases in land remaining under grass and suggests that during the first years the rate of increase is slow. The plots for which wireworm populations are recorded in Table 3 adjoined one another and the maintenance of a high population at one end of the series suggests that click beetles may not travel far from their emergence sites to lay eggs.

The writers acknowledge their indebtedness to Dr Morris Cohen and members of the Field Station staff and wireworm teams of the North-Western Province for the examination of soil and the recording of the wireworm populations.

TABLE 3. Wireworm populations per acre in ley plots

Plot	1938-9	1939-40	1940-1	1941-2	1942-3
A	22,000 ± 6,000	176,000 ± 31,000	—	776,000 ± 49,000	956,000 ± 57,000
B	Roots	Cereals	* 137,000 ± 44,000	284,000 ± 34,000	355,000 ± 26,000
C	Cereals	76,000 ± 13,000	35,000 ± 5,000	269,000 ± 29,000	282,000 ± 24,000
D	Cereals	86,000 ± 14,000	131,000 ± 19,000	194,000 ± 33,000	245,000 ± 24,000
E	Roots	Cereals	127,000 ± 20,000	221,000 ± 37,000	265,000 ± 29,000

* Based on 20 samples.

REFERENCES

- ANON. (1944). Wireworms and food production. *Bull. Minist. Agric., Lond.*, no. 128.
- GOUGH, H. C. & EVANS, A. C. (1942). Some notes on the biology of the click beetles, *Agriotes obscurus* L. and *A. sputator* L. *Ann. appl. Biol.* **29**, 275-79.
- LEES, A. D. (1943). On the behaviour of wireworms of the genus *Agriotes* Esch. (Coleoptera, Elateridae). *J. exp. Biol.* **20**, 43-60.
- MILES, H. W. (1942). Wireworms and agriculture, with special reference to *Agriotes obscurus* L. *Ann. appl. Biol.* **29**, 176-80.
- SALT, G. & HOLLICK, F. S. J. (1944). Studies of wireworm populations. *Ann. appl. Biol.* **31**, 52-64.
- STEWART, R. (1938). Observations on the soil of the Warburton Experimental Field. *Annu. J. R. Lancs. Agric. Soc.* p. 69.

(Received 7 February 1945)

Control of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber, by allyl isothiocyanate, the mustard oil of *Brassica nigra* L.

BY C. ELLENBY, B.Sc., PH.D., *Department of Zoology, King's College, Newcastle upon Tyne, University of Durham*

Previous work showed that, in the presence of root excretions of certain crucifers, larvae of the potato-root eelworm do not emerge from the cysts when stimulated by potato-root excretions; a similar lack of response is also shown in the presence of dilute solutions of allyl isothiocyanate, the mustard oil of black mustard. The present paper describes a small-scale field trial in which this mustard oil is applied with peat to the drills at the time of planting. An increase in yield of about 100% resulted from a dressing of the oil equivalent to 0.1 cwt./acre.

INTRODUCTION

In a recent paper (Ellenby, 1945) it was shown that, in the presence of root excretions from seedlings of white mustard, black mustard, and perhaps cress, potato-root excretions will not stimulate the emergence of larvae of the potato-root eelworm from the cysts; on return to potato-root excretion alone, normal emergence occurred. These results therefore extend the findings of Triffitt for white mustard (1929, 1930), who followed up the observations of Morgan (1925) that few cysts were formed on the roots of potato plants grown in infected soil together with white mustard plants. Field attempts, using as green manure white mustard (O'Brien & Prentice, 1930, 1931, and reported by MacM. 1932) and a variety of crucifers (Ellenby, unpubl.), were unsuccessful: the lack of success was thought to be due to the difficulty of applying the excretions in sufficient quantity in the field (MacM. 1932). That crucifers other than white mustard could also influence emergence in this way, and, more particularly, that dilute solutions of allyl isothiocyanate, the mustard oil of black mustard seed, were also effective (Ellenby, 1945), suggested that this difficulty might be overcome; the present paper describes a small-scale field trial of this mustard oil.

MATERIALS AND METHODS

Smedley (1939) carried out laboratory trials with a number of different isothiocyanates and found the phenyl form to be very effective in killing the contents of eelworm cysts; a fairly large-scale field trial with this isothiocyanate gave promising results. As already pointed out, however (Ellenby, 1945), this worker appears to have been unaware of the work of Morgan (1925) and Triffitt (1929, 1930) on the effect of white mustard on the reaction of eelworm larvae to potato-root excretion; her attention is therefore directed solely to the lethal action of the isothiocyanates. None of the isothiocyanates found in the mustards and related plants is tested in the laboratory; and in the field trial, phenyl isothiocyanate,

absorbed on to talc, is rototilled into the soil all over the treated plots 3 weeks before planting. This method of application to the soil may be suitable if lethal action only is considered; but if the reaction of the eelworm to potato-root excretion in the presence of mustard oil is to be considered, it may be more useful to apply the oil in the drill and at the time of planting.

Randomized block experiments were laid out on portions of each of two highly infested allotments, X and Y. X, a long strip, was laid out in four blocks, each of four rows of ten plants running at right angles to the length of the plot; one row for each of the four treatments, and 160 plants in all.

The following four treatments were applied to the drills at the time of planting:

- A. Artificial + water.
- B. Artificial + water + peat.
- C. Artificial + water + allyl isothiocyanate.
- D. Artificial + water + peat + allyl isothiocyanate.

Every row received a dressing of a general fertilizer at the rate of 1 oz./yd. of drill. A gallon of water was also applied to each row, either watered directly into the drill (A), or watered in after 3 c.c. of allyl isothiocyanate had been stirred up in it (C); in the case of the other two treatments, the water was thoroughly mixed with sufficient peat to absorb it, about 4 lb. (B), or mixed with the same quantity of peat after 3 c.c. of allyl isothiocyanate had been stirred up with it (D). As the peat was rather coarse, most of the oil would probably be held in solution and very little adsorbed on to the peat. Great Scot setts were planted at 1 ft. intervals in the rows, and, in order to prevent the interaction of the treatments, the rows were set 3 ft. 6 in. apart.

Plot Y was smaller; in order to have four replicates it was necessary to reduce the number of plants in the rows to five, at 1 ft. intervals; eighty plants in all. The four treatments in each block were as follows:

- (a) Artificial + water.
- (b) Artificial + water + 0.5 c.c. allyl isothiocyanate.
- (c) Artificial + water + 1 c.c. allyl isothiocyanate.
- (d) Artificial + water + 3 c.c. allyl isothiocyanate.

Artificial was applied at the same rate as in plot X; the mustard oil, however, was watered into the drills as in treatment C of plot X, but the rate of application was less, in two cases, and twice as great in the other case (d).

Observations were made during the season on the growth of the plants in the different rows; those treated with peat and mustard oil were noticeably better, particularly as the season advanced. The crop was harvested when the last of the plants had died down and the yield, to the nearest oz. per plant, recorded. No effort was made to separate the tubers into size categories.

in yield produced by this treatment in block 4 gives its mean value an unduly high value, at least, as far as its effect throughout the plot is concerned.

Table 2 shows that the yields in plot Y were uniformly low and roughly equal to the yields in the worst part of plot X. The error mean square, 84.2, is almost equal to the mean squares 'between blocks' and 'between treatments', 114 and 135.3 respectively; position and treatments are without significant effect. Although the yields for the three dosages are higher than control, the standard error for the difference between any two means, ± 6.49 , shows that in no case is the difference significant.

TABLE 1. Plot X. Total yield per ten plants (oz.)

Treatment per 10 plants	Blocks				Treatment means and S.E.*
	1	2	3	4	
Artificial + water (A)	43	49	84	107	70.75 ± 14.125
Artificial + water + peat (B)	31	41	77	122	67.75 ± 14.125
Artificial + water + allyl isothiocyanate 3 c.c. (C)	44	63	52	209	92.00 ± 14.125
Artificial + water + peat + allyl isothiocyanate 3 c.c. (D)	66	105	162	211	136.00 ± 14.125

* Based on error mean square.

TABLE 2. Plot Y. Total yield per five plants (oz.)

Treatment per 5 plants	Blocks				Treatment means and S.E.
	1	2	3	4	
Artificial + water (a)	15	13	6	24	14.50 ± 4.58
Artificial + water + allyl isothiocyanate 0.5 c.c. (b)	17	21	36	38	28.00 ± 4.58
Artificial + water + allyl isothiocyanate 1.0 c.c. (c)	20	12	44	24	25.00 ± 4.58
Artificial + water + allyl isothiocyanate 3 c.c. (d)	25	19	24	17	21.25 ± 4.58

RESULTS

The total yields, in oz. per row, are presented in Tables 1 and 2, for plots X and Y respectively. Table 1 shows the following: (1) The yield for each treatment decreases along the length of the allotment, i.e. from block to block. (2) In each block, the row treated with mustard oil and peat shows the highest yield; in three blocks, it is almost exactly 100% greater than the controls; in block 1, where the yields were the lowest, the increase is not so great. (3) None of the other treatments is effective; in block 4, however, where the yields are highest, the yield for mustard oil applied alone almost equals that for the combination of oil and peat.

An analysis of the variance shows significant effects for both positions and treatments; with an error mean square of 798.25, the variance ratio for treatments = 4.97 (p well below 0.05), and 13.05 (p almost 0.001) for positions. With a S.E. of ± 14.125 , the mean total yield for peat and oil (D) is significantly greater than for treatments A and B ($p < 0.02$, and p almost 0.01 respectively). The yield for treatment C, mustard oil alone, does not differ significantly from A and B, but it is almost significantly less than the yield for treatment D (p almost 0.05); the big increase

The results amplify those of plot X; mustard oil applied alone is evidently ineffective even at twice the dosage employed in plot X. However, under the similar conditions at the worse end of plot X, mustard oil and peat had a reduced effect; possibly, under less adverse conditions, the increased dose of oil, applied alone, may not be without effect; but it is clearly less efficient.

DISCUSSION

Clearly, the results show that, when the most suitable method and rate of application have been found, allyl isothiocyanate may prove very effective in controlling the potato-root eelworm. Already considerable improvement has been obtained by applying the mustard oil with peat; on the other hand, as expected, application directly to the soil was ineffective, except in one block where it was as good as the combined treatment.

The steady decline in yield along the length of plot X raises some interesting points. It is immaterial, for the moment, whether the decline is due to variation in degree of infection, or whether it is due to soil conditions, e.g. to the direction of drainage in the plot, but it is interesting to note that the yield

for the mustard oil and peat treatment shows almost exactly the same percentage increase over the controls in all blocks except that in which the control yield is lowest. Apparently, at the rate of application used in this work, mustard oil and peat may make a fair yield good and a poor yield reasonable; but it will only make a very poor yield less poor. Moreover, in block 4, which gave the highest control yields, yields which are still, however, on the low side, mustard oil alone, elsewhere ineffective, produced almost exactly the same increase as the oil and peat treatment. An understanding of these varied effects would probably go far to explain the mode of action of the isothiocyanate.

The yield for control plots in trials with phenyl isothiocyanate (Smedley, 1939) was 7.2 tons/acre; three rates of application were tried, $\frac{1}{4}$, 1, and 2 cwt./acre, the phenyl isothiocyanate being rototilled into the soil adsorbed on talc. An improvement in yield was found only in the case of the heaviest dressing. The present work was carried out under much less favourable conditions: control yields were considerably lower in most cases, though at the equivalent of 5-6 tons/acre at the better end of the plot, they approach the same order of magnitude; nevertheless, the yield was increased by 100% compared with the 35% increase reported by Smedley. Moreover, at normal planting distances, the rate of application would be equivalent to about 0.1 cwt./acre, compared with the 2 cwt. found effective with the phenyl form. Allyl isothiocyanate was thus 60 times as

effective, per pound weight; and it is even more effective per pound sterling, for it is considerably cheaper. But much of the greater effectiveness of allyl isothiocyanate may be due to the mode of application employed in the present work. Although allyl isothiocyanate is obtained from the seed of black mustard, it is phenethyl isothiocyanate which occurs in the roots of this and many other crucifers (Stahmann *et al.* 1943); both have the same fungicidal properties in solution, but the vapour of the former, due, apparently, to its lower vapour pressure, is many times more effective (Hooker *et al.* 1943). Low vapour pressure might also be an advantage in the soil; nevertheless, a comparison of these two oils and phenyl isothiocyanate, applied in the drills at the time of planting, would be of interest. However, the allyl form could be obtained from the seed of black mustard: this might be of decisive importance as it would presumably make it available at lower cost; already, for example, at '250 g.' prices, its use is almost economically possible.

My thanks are due to Prof. A. D. Hobson, for his interest and encouragement, to Messrs R. W. Elder and J. G. Harrison, for permitting me to carry out this work on portions of their infested allotments, and to the Hunters' Moor Allotments Association, for their co-operation on this and other occasions. A grant from the King's College Research Committee is gratefully acknowledged.

REFERENCES

- ELLENBY, C. (1945). The influence of crucifers and mustard oil on the emergence of larvae of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **32**, 67.
- HOOKE, W. J., WALKER, J. C. & SMITH, F. G. (1943). Toxicity of beta-phenethyl isothiocyanate to certain fungi. *Amer. J. Bot.* **30**, 632.
- MACM. (1932). Potato sickness and the eelworm *Heterodera schachtii*. *Notes Bur. agric. Parasit., St Albans*, no. 6.
- MORGAN, D. O. (1925). Investigations on eelworm in potatoes in South Lincolnshire. *J. Helminth.* **3**, 185.
- O'BRIEN, D. G. & PRENTICE, E. G. (1930). An eelworm disease of potatoes caused by *Heterodera schachtii*. *Scot. J. Agric.* **13**, 415.
- O'BRIEN, D. G. & PRENTICE, E. G. (1931). A nematode disease caused by *Heterodera schachtii* (Schmidt). *Bull. W. Scot. agric. Coll.* no. 2.
- SMEDLEY, E. M. (1939). Experiments in the use of isothiocyanates in the control of the potato strain of *Heterodera schachtii* (Schmidt). *J. Helminth.* **17**, 31.
- STAHMANN, M. A., LINK, K. P. & WALKER, J. C. (1943). Mustard oils in crucifers and their relation to resistance to clubroot. *J. agric. Res.* **67**, 49.
- TRIFFITT, M. J. (1929). Preliminary researches on mustard as a factor inhibiting cyst formation in *Heterodera schachtii*. *J. Helminth.* **7**, 81.
- (1930). On the bionomics of *Heterodera schachtii* on potatoes, with special reference to the influence of mustard on the escape of larvae from the cysts. *J. Helminth.* **8**, 19.

(Received 9 March 1945)

Studies upon the copper fungicides

VII. The solution of copper from dressings on the pea seed

BY R. L. WAIN AND E. H. WILKINSON

Agricultural and Horticultural Research Station, Long Ashton, Bristol

During the process of swelling pea seeds yielded substances to water which were capable of dissolving the insoluble copper dressings normally applied to the seed to prevent 'pre-emergence damping off'. This occurred under conditions in which soil factors were eliminated and irrespective of whether the dressing was on the surface of the seed or treated separately. Copper was readily taken into solution from dried 4:4:50 Bordeaux Mixture by water in which peas had been soaked, a circumstance which proved that the formation of soluble complex forms of copper was involved.

A chemical investigation on the solution obtained by soaking peas in water showed that volatile acids or their salts were present together with a protein-like material in colloidal solution. The occurrence of amino derivatives and other nitrogenous materials as well as carbohydrates was indicated, and evidence of the presence of asparagine and citrate was also obtained.

The suggestion is made that both the fungicidal and phytocidal actions of insoluble copper dressings on pea seed depend largely on the effect of the pea seed exudates, notably colloidal protein-like material and certain amino derivatives, in bringing copper into solution.

1. INTRODUCTORY

The disease known as 'pre-emergence damping-off' in peas is usually controlled by seed dressing with a protective fungicide. For this purpose insoluble copper compounds are most popular although organic mercury derivatives and organic fungicides are also employed (Brett *et al.* 1937; Felix, 1942). Copper protectants such as cuprous oxide (Horsfall, 1932; Ogilvie *et al.* 1939) and copper sebacate (Wain & Wilkinson, 1942, 1943*a*), although giving excellent control, may lead to copper damage especially under dry soil conditions (Ogilvie *et al.* 1939). Presumably both the fungicidal and phytocidal actions of these substances are related to the appearance of soluble copper. It was shown by McCallan & Wilcoxon (1936) and in Part VI of this series (Wain & Wilkinson, 1943*b*) that fungicidal action depends upon soluble copper produced under the influence of spore excretions and perhaps other agencies. With pea seed much soluble copper might be necessary to produce damage. How such concentrations are produced is uncertain, but Marten & Leach (1944) suggested that nitrogenous products of bacterial decomposition of proteins, known to be present in varying amounts in the soil, might influence the solubility of cuprous oxide to such an extent that damage is caused. These authors also showed that solutions of various amino-acid and protein materials dissolve much copper from cuprous oxide.

2. EXPERIMENTAL

Pea varieties. Peas of the var. Foremost, Surprise and Eclipse, harvested the previous season and visibly free from injury, were used throughout.

Copper salts. The preparation and analyses of the copper salts have already been described by Martin *et al.* (1942). The Bordeaux Mixture was also made as described in that paper. Glass distilled water was used in all this work.

Analysis. Soluble copper was estimated by the diethyldithiocarbamate colorimetric method, using the technique described by Wain & Wilkinson (1943*b*).

3. RESULTS

(a) *Copper dissolved from treated peas allowed to stand in water for 24 hr.*

Five lots of fifty peas (var. Foremost) were shaken with different copper compounds until evenly coated. Each group was then freed from superfluous dressing and placed in a flask with 100 ml. water. A control consisting of fifty untreated seeds in 100 ml. water was also included. At the same time a quantity of each dressing in excess of the amount adhering to the treated seeds was placed in separate flasks with 100 ml. water. After 24 hr. the liquids were removed, centrifuged and subjected to duplicate analyses for soluble copper. The treated seeds were placed on moist filter papers in enclosed Petri dishes and a germination count was made after 5 days (Table 1). In further experiments pea seeds were treated with copper oleate by immersing in a petroleum-ether solution of the substance and allowing the solvent to evaporate. On standing in Petri dishes on moist filter paper the visible deposit slowly disappeared and soluble copper was readily detected.

(b) *Copper dissolved from various dressings by water extracts of pea seeds*

Five hundred peas (var. Foremost), after washing in water, were submerged in 1 l. of water for 24 hr.: 50 ml. portions of the resulting liquid were then shaken with 0.5 g. quantities of various copper compounds for 24 hr. using a mechanical agitator with a rotary motion. It was observed that the resulting solutions, except in the cuprous thiocyanate experiment, were strongly blue in colour. After centrifuging, soluble copper in the solutions was determined (Table 2).

TABLE 1. *Copper dissolved from dressings on germinating pea seeds during 24 hr.*

Compound	p.p.m. Cu dissolved		Germination of treated seeds after 5 days
	By distilled water (av. of duplicates)	From compound on seed (av. of duplicates)	
Copper sebacate	4.35	18.42	90
Copper oxyfluoride	23.53	36.35	46
Copper oxychloride	3.05	15.60	100
Copper thiocyanate	0.66	1.38	100
Copper mucate	1.94	6.91	98

TABLE 2. *Copper dissolved from different compounds by water in which peas had been soaked for 24 hr.*

Compound	p.p.m. Cu dissolved (av. of duplicates)
Copper sebacate	37.59
Copper oxyfluoride	58.35
Copper oxychloride	34.19
Copper thiocyanate	3.30
Copper mucate	24.42

TABLE 3. *Copper dissolved from 4:4:50 Bordeaux Mixture deposit by water in presence of pea seed after 24 hr.*

Variety of pea	p.p.m. Cu dissolved (av. of duplicates)
Surprise	Exp. 1 27.3
	Exp. 2 31.2
Foremost	Exp. 1 50.6
	Exp. 2 49.1
Water alone	0.4

(c) *Copper dissolved from an excess of Bordeaux Mixture deposit by water in the presence of pea seed*

10 ml. portions of 4:4:50 Bordeaux Mixture were pipetted into 14 cm. Petri dishes and allowed to dry and carbonate. Duplicate lots of seventy-five peas of the vars. Foremost and Surprise were washed with water, placed in the dishes and allowed to stand with 150 ml. water for 24 hr. after which the liquids were removed, centrifuged and analysed. A blank was included by placing 150 ml. water only in a treated dish (Table 3).

4. A CHEMICAL INVESTIGATION ON THE EXUDATE OF PEAS (VAR. FOREMOST)

(a) *Salts of volatile acids*

Four thousand well-washed peas were soaked in 2½ l. of water for 17 hr. and the resulting liquid passed through a Seitz filter. The clear solution was made just alkaline to litmus and concentrated under reduced pressure at 40°C. The concentrate, after being made acid with sulphuric acid, was vigorously steam distilled and the steam distillate examined for volatile acids. Formic and acetic acids were readily detected by qualitative tests, the latter being isolated in the form of its *p*-phenyl phenacyl ester. The following results, all referred to 1000 peas, were also obtained:

Total volatile acids	≡ 9.36 c.c. N/10 NaOH
Formic acid	≡ 5.75 c.c. N/10 NaOH
Acids other than formic	≡ 4.05 c.c. N/10 NaOH

(b) *Other volatile constituents*

The distillate of the solution obtained by soaking 1000 washed peas for 17 hr. in 1 l. of water after Seitz filtration gave negative tests for alcohol, formaldehyde, acetaldehyde and acetone.

(c) *Main analysis*

Four lots of 5000 washed peas were each soaked in 3 l. of water for 17 hr. and the combined liquids were Seitz filtered. The clear amber-coloured liquid had a pH of 5.8 and a sp. gr. of 1.002 at 18.5°C.

TABLE 4. *Analyses on the concentrated solution of the exudate from peas (var. Foremost)*

	g. per 1000 peas
Dry matter	2.2620
Ash	0.6504
Total N (Kjeldahl)	0.0782
Amino-N (van Slyke)	0.0421
Total phosphate (as P ₂ O ₅)	0.0398
Reducing sugars (calc. as glucose)	0.6540
Reducing sugars after hydrolysis (calc. as glucose)	1.0410

The solution was concentrated at 40–50°C. under reduced pressure to about 4 l. During this process a greyish white solid separated in the flask suggesting that the clear filtrate contained material in colloidal solution. Although the solid separated readily on heating it was not precipitated by the usual flocculating salts. The precipitate was filtered off, washed well with water, alcohol and ether and dried at 100°C.; 8.943% N was found by the Kjeldahl method and phosphate was detected.

The main filtrate gave the results shown in Table 4. The ash on qualitative analysis showed sodium, potassium, calcium and phosphate.

The solution gave an immediate precipitate with neutral lead acetate solution, but a positive test for free amino acids, using ninhydrin reagent, was not obtained.

A portion of the main solution was treated successively with neutral and basic lead acetate solutions, and the precipitated lead salts were centrifuged and well washed with water. They were then suspended in water and decomposed with hydrogen sulphide. The precipitated lead sulphide was filtered off and the filtrate, after concentration under reduced pressure, was treated with an excess of mercuric nitrate solution, the resulting precipitate being centrifuged and well washed with water. After decomposition with hydrogen sulphide and the removal of the precipitated mercuric sulphide, a solution was obtained which dissolved cupric hydroxide to give a deep blue solution and which on warming with sodium hydroxide gave ammonia. These tests and the precipitation with mercuric nitrate solution indicated the presence of asparagine or glutamine (Schulze, 1884).

Attempts were made to isolate asparagine from the solution by the crystallization method of Chibnall (1924), but without success. The sticky brown oil obtained after evaporation to dryness was therefore treated with 3:5-dinitrobenzoyl chloride and sodium hydroxide as described by Saunders (1934). A product having a m.p. 184–189°C. was isolated in small quantity. This, on crystallization from water, appeared in the form of white plates, m.p. 192–195°C. Saunders (1934) obtained 3:5-dinitrobenzoyl *l*-asparagine in flat glistening leaflets, m.p. 196°C. The solution, after precipitation with lead acetate, was subjected to an extended qualitative treatment. Lactic, malic and malonic acids were absent, but citric acid was identified by conversion first to acetone dicarboxylic acid and finally acetone (Challenger *et al.* 1928).

5. DISCUSSION

Dressings of various insoluble copper compounds applied to pea seed, while giving protection from attack by soil fungi, may kill the seed by the formation of high local concentrations of soluble copper.

Apart from the work of Marten & Leach (1944) little attention has been given to the problem of how copper dissolves from insoluble seed dressings and, as far as the present writers are aware, interaction with substances exuded from the seed itself has not previously been examined. The results in Table 1 show that copper is dissolved from a variety of compounds on peas greatly in excess of the amounts yielded by these compounds to water under conditions free from the influence of soil factors. In this respect cuprous thiocyanate behaves anomalously, a result in agreement with the fungicidal (Martin *et al.* 1942) and spore exudate* data and its poor performance as a seed protectant (Wain & Wilkinson, 1942).

The results in Table 2 indicate that contact with the seed is unnecessary for the solution of copper

from the dressings, for copper is shown to dissolve in water in which peas had been previously soaked. This result supports the view that an exudate from the germinating pea is partly responsible for the production of soluble copper. If such a mechanism is operative, it would appear to be analogous to that by which fungal spores receive toxic concentrations of soluble copper from insoluble fungicides (Wain & Wilkinson, 1943*b*). Wain & Wilkinson (1943*b*) demonstrated that copper could dissolve from an excess of dried Bordeaux Mixture under the influence of spore extracts only by a mechanism involving complex formation. Since copper is dissolved from this fungicide by solutions of pea exudate (Table 3), it would appear that a complex formation is likewise involved. The formation of strongly blue-coloured solutions in the shaking experiments supports this view for, on addition of mineral acids to these solutions, the blue coloration was destroyed, a treatment which is known to convert complex copper ions to the relatively colourless cupric ion.

These observations suggest that the role of carbon dioxide, formed during the respiration of the seed, is unimportant in rendering the copper soluble.

To support the conclusion that the solution of copper is effected by complex formation, the chemical character of the pea exudate was examined. The acidity of the solution of pea exudate was not sufficiently marked to suggest that it is an important factor, while the volatile acids, being present in the solution mainly as salts, are unlikely to be solvents.

The high nitrogen content of the greyish white solid which separated from the heated solution indicates protein present in the original exudate in colloidal solution. As such substances are known to dissolve copper from insoluble fungicides (e.g. Marten & Leach, 1944), it may be assumed that this colloidal material is partly responsible for solution of copper from the dressings. The residual solution (Table 4) also contained nitrogenous material, and its relatively high proportion of amino-nitrogen indicates the presence of compounds capable of forming soluble complexes with copper. The carbohydrates in this solution (Table 4) would be inactive in this respect, but the activities of salts of certain hydroxy acids (Wain & Wilkinson, 1943*b*)—and citric acid was identified—must not be overlooked.

The intensive formation of asparagine in the seed of the germinating legume is well known (Chibnall, 1939), and in the present work this material was found in the exudate from peas. Solutions of this substance readily dissolve copper by a mechanism involving complex ion formation (Wain & Wilkinson, 1943*b*).

There is accordingly ample evidence of the presence, in the pea exudate, of substances capable of dissolving copper by complex formation and, of these materials, the amino and protein constituents are predominant. Such substances co-ordinate with

* Unpublished results.

copper to give complexes some of which may be dissociated in solution to yield a proportion of cupric ion (Wain & Wilkinson, 1943*b*). But if both complex and cupric ions can be present when copper dissolves from an insoluble dressing, the question arises, as in the case of toxicity to the fungal spore, which form of copper is more toxic to the pea seed? Experiments have been designed to throw light on this

point and the results will be communicated in a later paper.

The authors thank Dr H. Martin and Mr R. W. Marsh for their assistance in the preparation of this paper. The work was carried out with the aid of a grant from the Agricultural Research Council.

REFERENCES

- BRETT, C. C., DILLON-WESTON, W. A. R. & BOOER, J. R. (1937). Seed disinfection. III. Experiments on the germination of peas. Seed protection by the use of disinfectant dusts containing mercury. *J. agric. Sci.* **27**, 53.
- CHALLENGER, F., SUBRAMAMAM, V. & WALKER, T. K. (1928). The mechanism of the formation of citric and oxalic acids from sugars by *Aspergillus niger*. *J. chem. Soc.* p. 208.
- CHIBNALL, A. C. (1924). Nitrogenous metabolism of the higher plants. VI. Role of asparagine in the metabolism of the mature plant. *Biochem. J.* **18**, 399.
- (1939). *Protein Metabolism in the Plant*, p. 38. New Haven: Yale University Press.
- FELIX, E. L. (1942). Tetrachloro-*para*-benzoquinone, an effective organic seed protectant. *Phytopathology*, **32**, 4.
- HORSFALL, J. G. (1932). Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. *Bull. N.Y. St. agric. Exp. Sta.* no. 516.
- MCCALLAN, S. E. A. & WILCOXON, F. (1936). The action of fungus spores on Bordeaux mixture. *Contr. Boyce Thompson Inst.* **8**, 151.
- MARTEN, E. A. & LEACH, J. G. (1944). Some factors influencing the solubility of cuprous oxide in relation to its toxicity as a fungicide. *Phytopathology*, **34**, 459.
- MARTIN, H., WAIN, R. L. & WILKINSON, E. H. (1942). Studies upon the copper fungicides. V. A critical examination of the fungicidal value of copper compounds. *Ann. appl. Biol.* **29**, 412.
- OGILVIE, L., CROXALL, H. E. & HICKMAN, C. J. (1939). Cuprous oxide as a seed protectant. *Ann. Rep. Long Ashton Res. Sta.* p. 88.
- SAUNDERS, B. C. (1934). The identification of amino-acids by means of 3:5-dinitro-benzoyl chloride. *Biochem. J.* **28**, 580.
- SCHULZE, E. (1884). Detection of asparagine and glutamine in vegetable juices and extracts. Abstract in *J. chem. Soc.* p. 373.
- WAIN, R. L. & WILKINSON, E. H. (1942). Investigations with various copper compounds in relation to damping off in peas. *Ann. Rep. Long Ashton Res. Sta.* p. 59.
- (1943*a*). A new copper seed protectant. *Gdnrs' Chron.* **113**, 27.
- (1943*b*). Studies upon the copper fungicides. VI. The solution of copper from Bordeaux and Burgundy mixtures. *Ann. appl. Biol.* **30**, 379.

(Received 20 November 1944)

Studies upon the copper fungicides

VIII. The penetration of copper into germinating peas

By R. L. WAIN AND E. H. WILKINSON

Agricultural and Horticultural Research Station, Long Ashton, Bristol

Three varieties of pea seed showed less damage when treated with solutions of simple cupric salts than with solutions of equivalent strength in which the copper could occur in complex form. On adding supplementary pea exudate to copper sulphate solution, thereby converting some cupric to complex copper, greater damage was caused. In spite of the greater phytotoxicity of complex forms of copper, the copper intake by the seeds was less. Sealing of the micropyle had little effect on the water and copper intake of peas.

Colorimetric tissue tests on treated peas indicated a high concentration of copper in the testa and the embryonic radical with only small amounts in the cotyledonary tissues. These observations were largely confirmed by chemical analyses on the testas, cotyledons and embryos of copper treated peas.

The results suggest that when copper dissolves from dressings on pea seeds much becomes fixed on the testa, so providing fungicidal protection. The soluble complex forms of copper produced by pea exudate are markedly phytotoxic and injury to the embryonic radical is regarded as the main cause of damage to peas.

1. INTRODUCTORY

In Part VII of this series (Wain & Wilkinson, 1945) it was demonstrated that substances exuded from the germinating pea seed are involved in the solution of copper from the insoluble dressing normally applied to control 'pre-emergence damping-off', and further it was shown that such copper enters solution in complex form. But although the copper enters solution in this state it is not necessarily the complex which acts phytocidally, for in solution such complexes may be dissociated to yield cupric ions. This dissociation is governed by the stability constant (Wain & Wilkinson, 1943). With the fungus spore, evidence has been put forward that certain complex copper ions are not themselves the toxic agent but act as a bridge by which copper is transported to the spore wall. In agreement with this it was found that very stable complex forms of copper were relatively non-fungicidal.

Since there is a similarity between the action of the fungus spore and that of the germinating pea in producing soluble copper from insoluble fungicides, and in view of the results obtained in a study of the former problem, the mechanism by which damage is caused to peas was investigated.

2. MATERIALS AND METHODS

Pea varieties. Only peas free from visible injury were used of the vars. Foremost, Surprise and Eclipse.

Copper salts. These were prepared and analysed as described in Part V of this series (Martin *et al.* 1942).

Dissection of peas. A cut was made in the testa of the swollen pea using a sharp razor. With slight pressure the cotyledons with embryo could then be removed easily. The embryo was then detached from the cotyledons by severing the cotyledonary stalk.

Copper analysis. The determinations on whole and dissected peas, after ashing, extracting with dilute HCl and deionization of the iron, were made by the diethyldithiocarbamate method, using the technique described previously (Wain & Wilkinson, 1943).

Glass distilled water was used throughout.

3. RESULTS

(a) Germination of peas in solutions of simple and complex copper salts

The solutions of appropriate copper salts were made up at strengths equivalent to 50–500 p.p.m. Cu, and for each compound fifty peas and 50 ml. of solution at each concentration were used. From the germination results the concentration permitting 25% germination after 9 days was interpolated (Table 1).

Table 1 shows a generally increasing sensitivity to copper damage in the sequence Foremost—Surprise

—Eclipse, and a greater phytocidal effect of the copper complexes than of the simple salts.

In water alone the germination percentages were Foremost 89, Surprise 72, and Eclipse 46, and it was found that these figures were exceeded at the lower concentrations in the case of the sulphate, lactate and alaninate.

(b) Germination of peas in contact with copper sulphate solution and cupric oxide when supplementary pea excretions are present

A solution of pea exudate was obtained by soaking 400 seeds (var. Foremost) in 300 ml. water for 19 hr.

5 ml. quantities of copper sulphate solution containing 1000 p.p.m. Cu were added to 45 ml. of the exudate and to 45 ml. water in Petri dishes. This provided solutions containing 100 p.p.m. Cu. For the cupric oxide experiment, 1 g. of the substance was added to dishes containing 50 ml. pea exudate solution and 50 ml. water respectively. Controls containing 50 ml. pea exudate and 50 ml. water were also included.

Fifty peas (var. Foremost) were placed in each dish, the cover fitted and germination counts made at intervals up to 209 hr. The final results are given in Table 2 which also includes readings taken after 89 hr.

(c) Influence of supplementary pea exudate on the copper absorption of germinating peas

A solution of pea excretions was made by soaking 100 g. of well-washed pea seeds (var. Surprise) in 500 ml. water for 24 hr. The calculated amount of pure copper sulphate was then dissolved in 300 ml. of this liquid to provide a solution of 100 p.p.m. Cu.

Weighed lots of fifty peas (var. Surprise) were placed in three Petri dishes and 100 ml. of the above solution added to each so that the peas were completely submerged. At the same time three similar lots of peas were treated with the same volume of an aqueous copper sulphate solution also containing 100 p.p.m. Cu.

After standing for 23 hr. the seeds were removed, swirled rapidly for half-minute intervals in a beaker with three fresh lots of 200 ml. water, transferred to gold dishes and ashed. Copper estimations on the ash were then made, the results being given in Table 3.

(d) Copper absorbed by germinating peas in presence of solutions of various copper salts

Weighed lots of fifty peas (var. Surprise) were submerged in 100 ml. of solutions of copper salts at strengths corresponding to 10, 25 and 50 p.p.m. Cu. Each treatment was performed in duplicate and a control was also included.

After 23 hr. the peas were removed, washed as described above, ashed and the copper content determined (Table 4).

(e) *Water and copper intake of peas with open and closed micropyles*

(i) *Water intake.* Experiments in which untreated peas and peas having their micropyles closed with melted vaseline were allowed to soak in water for 18, 23 and 30 hr., showed that the percentage increase in weight of the seeds with the micropyles open was respectively 4.11, 4.38 and 7.74 greater than of those having their micropyles blocked.

corded. After 5 days those with sealed micropyles showed 19% germination against 7% in the group of untreated peas.

(f) *Copper absorption by different tissues of the pea seed*

Weighed quantities of 100 peas (var. Foremost) were submerged in 200 ml. of aqueous solutions of copper sulphate and copper glycinate containing

TABLE 1. *Copper concentrations permitting 25% germination of pea seeds after approximately 9 days*
p.p.m. Cu as

Variety	Sulphate	Lactate	Malonate	dl-Malate	Chloride	Alaninate	Glycinate	CuCl ₂ -ethylene-diamine	CuSO ₄ -ethylene-diamine
Foremost	390	410	260	—	—	190	140	—	—
Surprise	240	—	—	240	145	140	100	75	65
Eclipse	160	—	—	—	170	80	65	50	<50

TABLE 2. *Percentage germination of peas (var. Foremost) in the presence of copper compounds with and without supplementary pea exudate*

Treatment	% germination	
	After 89 hr.	After 209 hr.
CuSO ₄ + pea exudate (100 p.p.m. Cu)	34	80
CuSO ₄ + water (100 p.p.m. Cu)	74	100
CuO + pea exudate	32	48
CuO + water	62	82
Pea exudate	50	88
Water.	72	92

TABLE 3. *Copper absorption by peas (var. Surprise) after 23 hr. in presence of copper sulphate solution with and without additional pea exudate*

Treatment (100 p.p.m. Cu)		Total Cu absorbed mg. per 100 g. dry peas (av. of triplicates)
CuSO ₄ + exudate	Exp. 1	17.13
	Exp. 2	15.47
CuSO ₄ + water	Exp. 1	27.38
	Exp. 2	28.47

TABLE 4. *Copper intake of peas (var. Surprise) from solutions of copper salts after 23 hr.*

Copper salt	Conc. p.p.m. Cu	mg. Cu absorbed per 100 g. dry wt. of seed	Mean
Copper sulphate	10	4.37	4.37
	25	11.10	11.59
		12.08	
	50	24.13	20.97
		17.82	
Copper glycinate	10	3.59	3.65
		3.71	
	25	7.26	8.08
		8.89	
	50	15.86	17.03
Copper bis-ethylene-diamino sulphate		18.20	
	10	3.05	3.28
		3.51	
	25	5.01	7.01
		9.02	
Control	50	17.75	16.85
		15.84	
	—	—	0.95

(ii) *Copper intake.* Weighed lots of fifty peas (var. Foremost) with micropyles open and closed were submerged in solutions of copper sulphate at 300 p.p.m. Cu for 23 hr., the experiments being performed in duplicate. The seeds were then washed as described above, ashed and the copper determined. The peas with sealed micropyles contained an average of 90.32 mg. Cu per 100 g. dry weight, whereas the untreated peas contained an average of 82.85 mg. Cu per 100 g.

In this experiment parallel series of soaked peas after washing were placed in Petri dishes on moist filter papers and the percentage germination re-

100 p.p.m. Cu. Three lots were used with each compound and soaking was allowed to proceed for 23 hr. After washing, two sets were dissected to provide testas, cotyledons and embryos which were dried at 100°C. and the weights determined.

These separate tissues, along with the remaining sample of peas which was also dried at 100°C., were then ashed and the copper content determined.

A further experiment using copper sulphate and copper bis-ethylenediamino sulphate solutions at 200 p.p.m. Cu was also made.

The complete results from both experiments are given in Table 5.

4. DISCUSSION

As already pointed out, the phytocidal effect of copper on pea seeds can be due to the cupric ion or complex forms of copper, both of which are present in the solution produced when the insoluble dressing dissolves under the influence of pea excretions. The toxicity of solutions of copper salts ranging from copper sulphate, yielding only simple cupric ions to copper ethylenediamino sulphate and chloride which in solution yield a high proportion of complex ions, is indicated by the germination figures given in Table 1. With all three varieties it was found that the peas could tolerate much greater concentrations of cupric than complex ions. Thus, for example, with var. Surprise, germination was completely inhibited by treatment with copper sulphate solution containing 400 p.p.m. Cu, whereas 150 p.p.m. Cu supplied in the form of the ethylenediamino sulphate or

TABLE 5. *Distribution of copper in peas (var. Foremost) soaked in solutions of various copper salts for 23 hr.*

Material	mg. Cu per 100 g. oven-dry material (av. of duplicates)			
	Copper sulphate 100 p.p.m. Cu	Copper sulphate 200 p.p.m. Cu	Copper glycinate 100 p.p.m. Cu	Copper bis- ethylenediamino sulphate 200 p.p.m. Cu
Cotyledons	8.49	24.45	8.51	21.36
Embryos	70.11	286.4	64.72	211.5
Testas	263.4	286.7	132.6	97.0
Whole peas	31.42	51.46	24.38	—

chloride was equally effective. The observation that the germination figures at the lower concentrations of the simple salts were frequently higher than those in the controls is an example of the well-known phenomenon that small amounts of a toxicant may produce a stimulative effect.

It has already been indicated that cupric copper can be converted into more complex forms under the influence of excretions from pea seed. The results in Table 2 show that peas supplied with such complexes, formed by the interaction of pea excretions with copper sulphate or cupric oxide, were more readily killed than were peas not provided with supplementary excretions. This again indicates that complex forms of copper can be more toxic to peas than the simple cupric ion, and it will be noted from Table 3 that the greater damage to germination by the complex is caused by a smaller amount of copper entering the seed. Peas soaked in aqueous copper sulphate solution contained some 50% more copper than those soaked in copper sulphate with pea excretions.

The copper absorption from aqueous solutions of copper salts at equivalent strengths is shown in

Table 4, and here again an indication is given that complex copper ions are less readily absorbed than is the cupric ion.

It is generally understood that during the process of swelling prior to germination, water enters the pea seed through the micropyle and Croxall & Ogilvie (1939) have presented evidence that copper carried to the embryo by this route is the cause of phytotoxic action. In the present work in which the water and copper intake was determined on peas with sealed and unsealed micropyles, it was shown that absorption through the testa can readily occur. Both water and copper were absorbed freely by the peas with closed micropyles. The germination figures for peas with micropyles sealed and unsealed, after soaking in copper sulphate solution, indicate that the closing of the micropyle gives some protection against damage.

To investigate the distribution of absorbed copper in the pea seed, preliminary observations were made on several single seeds after soaking for 17 hr. in a solution of copper sulphate containing 1000 p.p.m. Cu. After washing, the testa was removed and the seeds cut in half so that the cut passed longitudinally through the embryo. On adding a drop of 2% aqueous sodium diethyldithiocarbamate solution, a brown coloration was produced in regions where copper was present. A high concentration was found in the meristematic layers of the embryonic radical, but little occurred in the embryonic plumule. The outer surface of the cotyledonary tissue was rich in copper, but the main bulk was comparatively free. The removed testa, in spite of the initial washing, contained much copper.

These preliminary observations were confirmed by the analytical results assembled in Table 5. These show that the testa generally retains much copper, though less was found when complex ions had been supplied than when cupric sulphate solution was used. It is probable that the cupric ion would be rapidly fixed by protein on coming into contact with the testa, whereas the bis-ethylenediaminocupric ion, for example, already very largely in complex form would not be fixed so readily.

The relatively high concentration of fixed copper in the testa, although possibly of low fungicidal value, might confer a prolonged protection against soil fungi.

The embryos (Table 5) contained large quantities of copper in all experiments, and it is not surprising, therefore, that copper damage can frequently occur. It is of interest to note that the preliminary experiments already described indicated that the embryonic radical contained the bulk of this copper, and it is here, therefore, that the seat of copper injury to peas is most likely to be found.

Again, in the preliminary experiments copper was only revealed on the outside layer of the cotyledons which explains the low quantities found in this

material by analysis (Table 5). Apparently absorbed copper is fixed by protein so that little can reach the interior of the seed. If so, it is most unlikely that any copper reaches the embryo by way of the cotyledons.

In actual practice, therefore, it is suggested that copper dissolved from dressings under the influence of an exudate from the pea becomes partly precipitated on the testa, which thereby receives a prolonged fungicidal protection. The copper passing through the testa in the region of the embryo and through the

micropyle, however, joins in building up a concentration on the embryonic radical which may be so high that the seed is killed.

The writers thank Dr H. Martin and Mr R. W. Marsh for help in the preparation of the paper. They are also indebted to Mr R. F. Batt for assistance with the analytical work. The work was carried out with the aid of a grant from the Agricultural Research Council.

REFERENCES

- CROXALL, H. E. & OGILVIE, L. (1939). The incorporation of growth hormones in seed dressing. *J. Pomol.* **17**, 362.
- MARTIN, H., WAIN, R. L. & WILKINSON, E. H. (1942). Studies upon the copper fungicides. V. A critical examination of the fungicidal value of copper compounds. *Ann. appl. Biol.* **29**, 412.
- WAIN, R. L. & WILKINSON, E. H. (1943). Studies upon the copper fungicides. VI. The solution of copper from Bordeaux and Burgundy mixtures. *Ann. appl. Biol.* **30**, 379.
- (1945). Studies upon the copper fungicides. VII. The solution of copper from dressings on the pea seed. *Ann. appl. Biol.* **32**, 240.

(Received 20 November 1944)

The mode of action of sheep dips

By E. T. BURTT, M.A., PH.D., *Agricultural Research Council Unit of Insect Physiology, London School of Hygiene and Tropical Medicine*

A study was made of the action on the tick (*Ixodes ricinus* L.) of some toxic substances used in dips. Methods are described of attaching ticks to limited areas on the sheep and of suspending ticks for individual tests.

Chemical potentials rather than concentrations should be used in comparing the toxicity of different solutions. Phenol is less soluble in P31 paraffin oil than in water, and a solution of phenol in P31 paralyses the tick more rapidly than the same concentration in water. Solutions of phenol in paraffin and water which are in equilibrium show the same effect on the tick. The potentials are the same, but the concentrations are different.

Each toxic substance tested acts more rapidly at a given concentration in a medium in which it is less soluble. Acid solutions of arsenious acid act more rapidly than alkaline. Solid arsenious oxide as crystals or as a deposit on wool acts more slowly than sodium arsenite under the same conditions, owing probably to its slow rate of solution. Sodium arsenite and arsenious oxide readily penetrate the tips of the feet, and sodium arsenite readily penetrates the dorsal surface of the tick.

Previous work on arsenical dips and the contact toxicity of wool dipped in sodium arsenite indicate that arsenic is a contact and not a stomach poison to ticks.

Phenols are toxic at low concentrations in water, but their greater solubility in wool fat and rapid evaporation from thin films explain their ineffectiveness as dips.

DDT (2, 2 bis-*p*-chlor phenyl 1, 1, 1, trichlorethane) penetrates the feet of the tick more readily than the rest of the body surface. DDT acts more rapidly at 1% in paraffin oil (solubility 1-3%) than in sesame oil (solubility 10%). Saturated solutions have the same effect. Similarly, vaseline solutions are more effective than solutions in lanoline.

Rotenone penetrates the feet of ticks more rapidly than the back. Larval ticks are more rapidly intoxicated by 0.1% solutions in olive oil (solubility 0.2%) than 0.1% solutions in castor oil (solubility 1-2%). Saturated solutions have the same effect.

The importance of a knowledge of the site of entry of insecticides into the organism is stressed.

The less soluble the substance in the medium, the less is needed to give a high chemical potential and the more rapid will be the penetration of the organism. With low solubility, however, a small loss by diffusion causes a large fall in potential. Solubility is analogous to capacity. In practice, media will be needed in which the toxic substance is neither so soluble that a wasteful amount is needed to produce a high potential nor so insoluble that losses by diffusion cause large falls in potential.

The carrier activity of different oil bases for insecticides and the synergistic action of insecticides applied together may in part be due to changes in solubility and hence chemical potential.

1. INTRODUCTION

The aim of this work is to give an understanding of the action of dips against the sheep tick *Ixodes ricinus* L. (Acarina: Ixodidae). The physical state in which the different poisons act on the tick and the route by which they enter the tick's body will be considered. One type of insecticide (a dip) and one organism (the sheep tick) will mainly be dealt with, but matters applying to insecticides in general will also be discussed.

Ixodes ricinus L. attaches to a vertebrate host once in each of the three instars larva, nymph and adult, and remains attached for 2-10 days depending on the instar. The usual sites for attachment are the face, ears, neck, axillae, groin and feet.

Consider the process of dipping a sheep infested with *Ixodes ricinus* L. (for more practical aspects of dipping see Milne, 1945). The sheep goes into the bath for, say, 1 min. at most: during this time the ticks meet the toxic agent at dip concentration. Then the sheep climbs out of the bath wet to the skin and starts to dry. The time taken for drying will vary with wind, sun and humidity, but 8 hr. is a rough estimate. The fate of the toxic agent during this time will depend on its relative solubility in water and the grease covering the wool fibres.

With a water-soluble substance like sodium arsenite the tick will meet during the drying time an increasing concentration ending with either a saturated solution or crystals of the salt, depending on the humidity of the fleece. Davies & Hobson (1935) have shown that the fleece usually has a low humidity varying from 40 to 80% relative humidity at the shoulder, 45-56% on the back and 65-100% on the rump. I found 55% relative humidity shown on a hair hygrometer against 90% in the air. Wool fat readily makes water in oil emulsions, and thus water-soluble substances may form such emulsions where the humidity is high.

Oil-soluble substances such as DDT will pass into solution in the wool fat and in this form will spread easily over the tick's body as the following experiment shows. Two female ticks are taken just after emergence from the nymphal skin, one is put for 10 min. on a sheep and their appearance is compared under the microscope. The tick from the sheep has a more glistening appearance due to wool fat having spread over it. This result is to be expected, since wool grease extracted from the fleece becomes fluid at about 35°C.; the temperature of the face and ears of a sheep was 35-37°C., and that of the back of an attached female tick was 32-34°C. (taken with a thermocouple in a room at 17°C.).

There are two aspects to the efficiency of a sheep dip: effectiveness as a killer of ticks attached at the time of dipping, and effectiveness as a means of preventing ticks from attaching after dipping. The two effects do not necessarily run parallel when

different substances are compared as dips. Thus sodium arsenite is a certain killer of attached ticks and DDT is a less certain killer of attached ticks (Heath & Mitchell, unpublished), yet the same DDT dip may give a better protection following dipping than the arsenical. A dip containing derris, on the other hand, gives as good an initial kill as an arsenical and yet confers greater protection against reinfestation (Milne, 1945). Attempts to improve sheep dips aim mainly at giving a lasting protection to the sheep. Sodium arsenite acts so well as an initial killer that there is less need for improvement in this direction, except perhaps in substituting for arsenic a substance less toxic to man and domestic animals.

2. MATERIALS AND METHODS

(i) Attachment of ticks to sheep

Dr H. H. Green very kindly loaned one-year-old Cheviot wethers for experiments at the London School of Hygiene. These sheep were used for the patch tests and provided the wool for *in vitro* tests. Downing (1936) uses a method for confining sheep scab, which has been followed for ticks. It consists in shaving or clipping a patch of wool on the back of the sheep between the shoulder and tenth ribs to one side of the vertebral column. A box is then stuck on to the skin with a cement of nine parts resin and one part beeswax heated to 100°C. This method is convenient for feeding small lots of ticks or for testing patches treated with dips. A box the same size as Downing employed (1½ in. internal diameter) can be used, but it is better to make one with a screw top from the cap of a petrol tin. The centre of the top is cut out and a wire gauze soldered in. The boxes will stay on for a period amply sufficient to allow adult female ticks to engorge.

Ticks do not often attach to the back in nature, but the natural regions of infestation are neither so easy to attach ticks to nor so easily examined once ticks are attached. The surface temperature and length of wool in clipped areas of the back approximate to those of the face and ears where ticks attach naturally.

(ii) Suspension of individual ticks

The head of a long pin was turned back on its shaft by two right-angled bends, and then dipped in rubber solution and brought into contact with the scutum of the tick, when it adhered and the tick could be lifted off its feet. Ticks could be removed from the pin, allowed to crawl about and then put on again without injury. The adhesion was strong enough to allow the tick's feet to be brought into contact with a substratum without the tick being able to break away.

(iii) Reagents

Phenol was estimated by the method given by Allen (1925, p. 281), in which the phenol is bromi-

nated and the excess bromine estimated iodometrically. A known solution of the phenol was titrated to determine the reaction.

P31 paraffin oil has the following specification: sp. gr. 0.856 (16° C.); initial b.p. 300° C. (range 50% at 358° C.; 85% at 374° C.); viscosity (redwood I) 140 sec. at 22° C.; unsulphonatable residue about 99%.

B.D.H. anhydrous lanoline was used. Wool fat can be considered in two fractions. Hobson (1936) extracts wool with ether and calls the residue left by evaporating the ethereal solution 'wool grease'. He again extracts the wool with water and refers to the residue from evaporating the aqueous extract as 'suint'. Pure lanoline is wool grease purified and dried and contains no suint.

The DDT used was a Geigy pure sample. The rotenone was a pure sample. Otherwise B.D.H. analytical reagents were used.

3. LABORATORY TESTING OF SUBSTANCES FOR USE AS A SHEEP DIP

Substances to be used as dips should either make toxic solutions in water, render the fleece toxic after dipping, or do both these things. In the first case the poison is in solution in water and, in the second, it is either a solid or a solution in wool grease.

I shall show that the substances which make effective sheep dips act as contact poisons. The system is essentially one in which a solution of the poison is brought into contact with the cuticle of the tick; the poison then passes from the solution into the tick and in time an effect on the tick is produced. We need to know how easily the poison leaves the solution it is in and passes into the tick; we are dealing with the same property of solutions which in rather different circumstances appears as osmotic pressure, which we can regard as an attraction between the solvent and the dissolved substance. The usual way of comparing the toxicity of two substances is to compare the effects on the organism of a range of concentrations of each substance in a given solvent. In dilute solutions the osmotic pressure is directly proportional to the concentration, but in concentrated solutions the osmotic pressure is greater than the concentration would lead one to expect, as Berkeley & Hartley's data (1906) for cane-sugar solutions show. These considerations apply even more forcibly to salts: in dilute solutions osmotic effects are directly proportional to concentration, but in concentrated solutions this is no longer true. Thus, in a concentrated solution of calcium chloride in water, the osmotic effects are greater than would be expected even supposing that the salt were completely ionized, i.e. 'the activity coefficient' is greater than unity.

Other difficulties arise when solutions of the poison in different solvents are compared, and here

the work of Ferguson (1939) is useful. He discusses the decrease in the toxic dose which is usually found in the lower compared with the higher members of a homologous series of organic compounds. He quotes data for the effect of aliphatic alcohols on the development of sea-urchin eggs, killing of bacteria, fumigation of red spider and the haemolysis of blood. The higher alcohols are effective at a smaller dose in each case. Ferguson points out that the solubility of the higher homologues is lower, and suggests that a better comparison is obtained by dealing not with absolute concentrations but with what he refers to as 'activity' or 'chemical potential'. This is roughly measured by the fraction

$$\frac{\text{concentration in the medium}}{\text{concentration required to saturate the medium}}$$

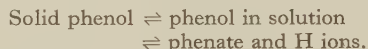
Ferguson examines the data of Tattersfield & Roberts (1920) for fumigation tests on the wire-worm. He shows that some inert substances toxic at low concentrations as vapours yet require that the medium (air) be nearly saturated: in other words, the partial pressure must be high. The same considerations apply to substances in solution. To obtain a clear idea of the toxicity of a substance in a medium we must know its solubility in that medium; the concentration alone may be misleading.

The results in Table 1 show this principle in operation. Phenol is more soluble in water than in P31 oil; at 23° C. a 0.134% solution in water is in equilibrium with a 0.0144% solution in P31 (distribution coefficient 9.3/1.0). The unfed larvae were immersed in the solutions in small glass dishes, removed at intervals and their condition noted. The chemical potential in the solutions at equilibrium will be the same. The rate of paralysis of the larvae is also the same (Table 1 (b) and (c)). On the other hand, a 0.134% solution of phenol in P31 (Table 1 (a)) acts from 5-10 times as fast as the same concentration in water. In this case the concentrations are the same, but the potential in the P31 oil solution is higher because phenol is less soluble in that medium.

The action of phenol can also be influenced by using an alkaline in place of a neutral solution. Table 1 (d) shows that phenol to which enough sodium hydroxide has been added to convert it into sodium phenate is 3-4 times slower in paralysing larvae immersed in it than the same concentration of phenol in water. This is explained in two ways difficult to separate which are, I think, different ways of looking at the same thing. Sodium hydroxide will convert the weak acid phenol into its salt, sodium phenate; the latter will be largely dissociated in dilute solution so that the phenol will be present mainly as phenate ions. In the neutral solution phenol will be present mainly as undissociated phenol. We might attribute the difference between (c) and (d) in Table 1 to the phenate ions penetrating

more slowly than undissociated phenol. This is the same explanation as Richardson & Shepard (1930) give for their finding that free nicotine base paralyzes mosquito larvae 5-7 times as fast as the salt at the same concentration. Hoskins (1932) has done similar work comparing the effects of arsenious acid and sodium arsenite on the mosquito pupa.

Alternatively, sodium phenate is more soluble than phenol in water, in which case the situation is comparable to that when solutions of phenol in P31 and water are compared. Consider the following equations:



Addition of sodium hydroxide will on the ionic view take up the phenate ions and shift the reaction to the right so that more phenol passes into solution

712 eggs per female, but none of the eggs hatched. One hundred and five controls averaged 3190 eggs per female, and of these 99% hatched. Sodium arsenite had some effect on fertility, and since the females were already engorged when immersed, Ransom & Graybill conclude that arsenic can enter the tick through the cuticle as well as by the act of engorging.

Brunnich & Smith (1914) suggest that arsenic is absorbed mainly through the cuticle both during and after dipping. They noted that attached ticks were unharmed on any area of the host not immersed, showing that ticks do not get the arsenic from the blood.

Cooper & Laws (1915) state that 'experimental enquiry tends to show that the poison (i.e. arsenic) is imbibed by the tick while feeding on the host', and consider that 'the fact that females are rendered in-

TABLE 1. *Effect of solutions of phenol in paraffin and water on unfed larvae of Ixodes ricinus L. at 23° C.*

Conc. of phenol	Condition of larvae at time stated			
	5 min.	7½ min.	10 min.	
(a) 0.134% in P31	15 can progress 17 paralysed	9 can progress 23 paralysed	— 32 paralysed	
	30 min.	50 min.	60 min.	75 min.
(b) 0.0144% in P31	45 can progress 25 paralysed	27 can progress 43 paralysed	8 can progress 62 paralysed	— 70 paralysed
(c) 0.134% in water	38 can progress 12 paralysed	15 can progress 35 paralysed	5 can progress 45 paralysed	— 50 paralysed
	60 min	120 min.	210 min.	270 min.
(d) 0.134% in water as sodium phenate	45 can progress 8 paralysed	40 can progress 13 paralysed	16 can progress 37 paralysed	0 can progress 53 paralysed

(assuming that the amount of undissociated phenol in solution remains more or less constant).

Any treatment which increases the ionization of the phenol also increases its solubility, so that the two effects are not easily separable.

Different solutions of phenol are favourable for the study of the relationship between solubility and rate of toxic action. The effect of media of different solubility will be considered for each toxic substance discussed in this paper, although it is only with phenol that chemical data are so easily worked out.

3. THE ACTION OF SODIUM ARSENITE ON TICKS

(i) *How arsenic enters the tick*

The effectiveness of sodium arsenite as a killer of ticks is a fact of experience. Watkins-Pitchford (1911) found that only sixty-nine adult females survived dipping out of 10,000 females counted.

There has been some discussion on how sodium arsenite enters the tick. Ransom & Graybill (1912) tested various arsenical dips on cattle ticks (*Margaropus annulatus* Say). One hundred and twenty-three gorged female ticks were immersed for 2 min. in sodium arsenite solution; 82% laid eggs averaging

fertile by immersion in the dipping fluid can have little effect in actual practice'.

Cooper & Laws based their conclusion to a great extent on the work of Watkins-Pitchford (1911). The latter considered that ticks were killed partly by absorbing arsenic through the cuticle and partly by sucking arsenic from the deeper layers of the mammal's skin. He estimated the arsenic in the hair and scurf, compared it with that in the skin itself, and showed that the total in the hair and scurf was greater than in the skin. However, he thought that 'the mere presence of arsenic in the hair itself has but small effect on the tick'. He states: 'If in an habituated animal a patch of skin is shaved closely and thoroughly washed so as to remove all deposited arsenic with the hair and epithelium before attaching the ticks, the lethal result will follow to the same degree as in the case of an habituated animal in which such precautions are not taken.' No details of this experiment are given, but it seems to be the basis for his conclusion that the arsenic on the hair has little effect on the tick, and that absorption of arsenic through the tick's cuticle only takes place in the dipping bath.

The latter view is rendered unlikely by the work of

Green & Dijkman (1918) who showed that Watkins-Pitchford's data for the total arsenic in the skin of regularly dipped cattle were too high by a factor of 40-60, and further that the ratio of arsenic in the hair and scurf to arsenic in the epidermis and dermis was much higher than Watkins-Pitchford had found (i.e. there was very little arsenic in the deeper layers of the skin). The concentrations from a hide 1 day after dipping were 0.85 g./sq.ft. in the hair and scurf, and 0.0041 g./sq.ft. in the deeper part of the skin. From this one suspects that the arsenic in the skin is less likely to be effective than that on the scurf and hair.

The data so far considered refer to cattle; but I think even on cattle with their short hair that ticks would be in contact with a watery solution for a longer period than the 2 min. which Ransom & Graybill's tests assume. Further, the concentration of arsenic will increase as the animal dries. The effect of a long period and a more concentrated solution is shown by the following experiment:

Groups of twenty unfed nymphs of *Ixodes*

of each tick containing the arsenite solution. Ten more ticks served as controls with a crystal of sodium chloride held on with rubber solution. After 6 hr. the arsenic ticks could still move, but were rather sluggish, and after 24 hr. all were dead: the controls were unaffected. We see then that arsenite will penetrate the dorsal cuticle of ticks. It is recorded that absorption of an arsenic and soap mixture took place quite readily through the cuticle of the gorged cattle tick *Boophilus annulatus* Aust. (Entomological Investigations, 1940-1).

Sodium arsenite was also applied to the tips of the legs only: five females were attached to pins with rubber solution and their feet were made to touch a film of sodium arsenite at 23° C. Two could no longer walk in 4 hr., the remaining three could no longer walk in 5½ hr. and all five were dead in 24 hr.

It is possible that the legs carried the poison on to the body. Five females were attached in such a way that the last two legs of one side were held out of all contact with the body by a band of rubber solution, and sodium arsenite applied to the ends. In 6 hr.

TABLE 2. *Absorption of arsenic by ticks with the proboscis blocked*

Solution	Conditions of ticks at		
	6 hr.	24 hr.	48 hr.
0.2 % sodium arsenite	5 ♀ alive	3 ♀ alive	5 ♀ dead
	5 ♂ alive	2 ♀ dead	2 ♂ alive
		3 ♂ alive	3 ♂ dead
		2 ♂ dead	
2.0 % sodium arsenite	5 ♀ much affected	All dead	—
	5 ♂ much affected	All dead	—
Pure water	—	—	5 ♀ and 5 ♂ alive

ricinus L. were put among glass-wool moistened with the following:

	6 hr.	24 hr.
0.22 % sodium arsenite	20 alive	8 alive, 12 dead
2.0 % sodium arsenite	6 alive, 14 dead	20 dead
Pure water	20 alive	20 alive.

It is possible that arsenic has entered through the mouth. The nymphs are rather small for manipulation, so the proboscis of adult males and females was blocked by a drop of cellulose paint applied to its tip. Groups of five males and five females were put among the solutions shown in Table 2.

Evidently ticks can be killed by long immersion in sodium arsenite which must enter by some route other than the proboscis.

When the sheep dries solid sodium arsenite, or a saturated solution, will be present in the fleece. A crystal of sodium arsenite (about 0.55 mg.) was put on the back of each of ten unfed female ticks, the crystal was moistened and then covered with rubber solution. The effect was to make a blister on the back

two were affected and all were dead in 24 hr. Sodium arsenite can thus readily penetrate the feet.

The above agrees with the findings of other workers for different arthropods. Woodworth (1938) immersed wireworms in sodium arsenite and found the salt in large quantity in the blood but not in the digestive tract. Hoskins (1932) found that mosquito pupae were killed by immersion in arsenious acid, and O'Kane & Glover (1935, 1936) showed that solid sodium arsenite penetrated the thoracic cuticle of the American cockroach.

(ii) *Toxicity of wool dipped in sodium arsenite*

An analysis of the mode of action of arsenic more related to practical conditions is to put ticks among locks of wool which have been dipped in sodium arsenite and allowed to dry. I am indebted to G. B. S. Heath for kindly analysing these samples while he was working in the Biochemistry Department at the Veterinary Laboratory, Weybridge.

A lock of fresh sheep's wool dipped in sodium arsenite and allowed to dry contained 3.7 mg. of As_2O_3 /g. of wool which is about the same as Heath

(unpublished) finds in the fleece of recently dipped sheep. The wool was conditioned at 100% relative humidity to prevent the ticks being desiccated and twenty nymphs were put with it. After 6 hr. two were dead, after 24 hr. sixteen were dead and after 48 hr. all were dead. A control lock of wool which had been dipped in water had no effect. Very greasy fresh wool will sometimes kill ticks put with it; but this power is lost after immersion in water, making it clear that the deaths in the arsenic wool are due to the contact effect of sodium arsenite.

The foregoing evidence suggests that ticks will be poisoned by the arsenic in the hair and scurf of the dipped animal. We have still to consider the experiment of Watkins-Pitchford in which he claims that the toxicity of the skin of a dipped animal (ox) cannot be removed by washing. I repeated this on the sheep as follows: the wool was clipped from six circular patches each about 4 in. across on the sheep's back and the fibres were left about 0.5 cm. long; 4 c.c. of 0.2% sodium arsenite was rubbed on to each of these patches on two successive days and allowed to dry.

TABLE 3. *Effect on female ticks of attachment to skin treated with sodium arsenite and the same skin subsequently washed*

No. of test	Arsenic patch	mg. $\text{As}_2\text{O}_3/\text{g. wool}$	Washed patch	mg. $\text{As}_2\text{O}_3/\text{g. wool}$
1	4/5 attached. 1 died in 2 days; 2 died in 3, and 1 in 4 days	1.6	4/5 gorged normally	1.0
2	5/5 attached. 5 died after 2 days	2.7	5/5 attached. 2 died after 8 days. 3 gorged normally	0.6
3	2/5 attached. 2 died after 2 days	—	5/5 gorged normally	—

The aim was to imitate the skin of an animal which had been frequently dipped. Three of the patches were washed with soap and water, allowed to dry, and then five female ticks were confined in boxes on each patch. Dr H. H. Green very kindly analysed samples of wool for arsenic from the first two pairs of boxes. The amount found compares satisfactorily with that found by Heath (unpublished) for recently dipped sheep. The fate of the ticks is shown in Table 3.

The washed patch was harmless to ticks except for a slight effect in test 2, where two attached females died in 8 days. In the arsenite patches no ticks engorged and no attached female lived longer than 4 days.

This test shows that arsenite can kill ticks after they have attached: a fact well known to field workers, which is, I think, the basis of the belief (e.g. MacLeod, 1941) that arsenic is a stomach poison. It is supposed that the tick must bite the sheep before it can be poisoned. Instead of this I suggest the following explanation. Arsenic exerts a gradual effect, and the first sign of poisoning is to slow the tick's movements rather than to disorganize them. Before attachment the tick picks up a dose of

arsenic which will ultimately kill it, but not before it has attached. If the tick took up arsenic by engorging, it is difficult to understand how arsenic, which is mainly on the surface of the skin, contrives to get past the tightly embedded proboscis and so into the blood which the tick is sucking up.

(iii) *The action of arsenic solutions of different pH*

Prof. J. Gray suggested that arsenical dips containing unionized arsenious acid would penetrate the tick more readily than dips of the same concentration in which the arsenic was ionised. The work of Hoskins (1932) on mosquito pupae showed that arsenious acid was more rapid in action than sodium arsenite of the same concentration, and that the difference of pH alone was not toxic. Using 0.01 molar As_2O_3 at pH 5 (where the arsenic is mainly in the form of arsenious acid) he got a 50% kill in 181 ± 8 min., using the same concentration at pH 11; where it is mainly in the form of arsenite ions, he found 800 ± 40 min. were required for 50% kill. Hoskins explains his results by the degree of ionisa-

tion of the arsenious acid, which as a weak acid in the presence of its salt obeys the equation

$$\text{pH} = \text{p}K_a + \log. \frac{\text{base}}{\text{acid}}$$

Hoskins took 2.10×10^{-8} for the dissociation constant of arsenious acid, or $\text{p}K_a = 7.22$.

Larval ticks were immersed in the arsenite solutions shown in Table 4 which all contained 0.2% As_2O_3 by titration: pH was determined to within 0.2 by indicators. It was surprising to find no difference between pH 8.9 and 2.0, for one would expect a change at pH values much above 7.22; however, the effect on ticks was slower at pH 11. Hoskins took pH's of 5 and 11, well to each side of the pK value. The value of K_a used by Hoskins is that of Zawidzki which was shown by Wood (1908) to be too high. Wood deduced a dissociation constant of 6×10^{-10} for arsenious acid from the effect of sodium arsenite on the rate of hydrolysis of methyl acetate. He also found $K_a = 26.5 \times 10^{-10}$ by measuring the conductivity of aqueous arsenious oxide solution. The latter is higher than his first value, but less than Zawidzki's. Wood attributed the discrepancy to impurities in the

arsenious oxide. Landolt-Bornstein (1927) gives 6×10^{-10} .

Wood's value of 6×10^{-10} gives $pK=9.22$ for arsenious acid. At pH 8.9, therefore, the arsenic will be as acid to ions in the ratio 2.1/1. These results are similar to those of Hoskins: the larval tick has behaved as a crude indicator for the titration of arsenious acid.

The difference in the rate of action of arsenious acid in alkaline and acid solution could equally well be attributed to the difference in solubility in the two media. Wood (1908) shows that adding hydrochloric acid to a solution of arsenious oxide lowers the solubility which on the ionic theory will be attributed to the effect of the excess of H ions in depressing the ionization of the arsenious acid. There are no published data for the effect of alkali on the solubility of arsenious oxide, but the solubility must be increased. The effect of sodium hydroxide can be regarded either as producing the readily soluble sodium arsenite or as increasing the fraction of the acid present as arsenite ions.

Ten females treated with arsenious oxide showed no effect after 3 days (cf. all dead in 24 hr. with sodium arsenite). Ten females were attached to pins and the feet only brought in contact with a layer of arsenious oxide: all could still progress after 8 hr. but none after 24 hr., although they could still move their legs (cf. none could walk after $5\frac{1}{2}$ hr. with sodium arsenite). O'Kane & Glover (1935, 1936) found a similar slowness of action of solid arsenious oxide as compared with sodium arsenite when these substances were put in cells on the back of the thorax of the American cockroach. I think the explanation lies in the fact that sodium arsenite very rapidly dissolves in any water present and subjects the tick to the action of a saturated solution, while arsenious oxide takes a long time to form a saturated solution in water.

To sum up the effects of arsenite solutions of different pH: acid solutions are quicker than alkaline in their action on the tick, but alkaline solutions confer a more rapid action on the wool dipped in them.

TABLE 4. *Effect of arsenious oxide solutions of differing pH on unfed larval ticks (2 hr. immersion) at 23° C.*

Solution	Test 1	Test 2
0.4% sulphuric acid	55 can progress 0 affected	57 can progress 0 affected
0.2% sodium arsenite plus 0.4% sulphuric acid, pH 2.0	23 can progress 25 moribund	20 can progress 24 moribund
0.2% sodium arsenite, pH 8.9	20 can progress 31 moribund	26 can progress 33 moribund
0.2% sodium arsenite plus 0.4% NaOH, pH over 11	46 can progress 28 moribund	33 can progress 14 moribund

There is a further aspect of arsenical dips of high and low pH; namely, the toxicity conferred on the wools immersed in them. Samples of wool were immersed in 0.2% As_2O_3 at pH 2.0 and 11.0 and allowed to dry. Two groups of four locks of wool were so treated, and Mr G. B. S. Heath very kindly analysed the dipped locks for arsenic. Two were selected in which the concentration of arsenic was nearly the same and unfed nymphs were put with them at 23° C.

Table 5 shows that the alkaline-dipped locks gave a quicker kill than the acid, since at 24 hr. only five out of a total of twenty-six were alive in the first case, while twenty out of twenty-six were alive in the second case. Further, in the first case sixteen of the twenty-one affected were dead, and in the second case the six affected were only moribund.

The explanation of the above difference is found by comparing the effect of solid sodium arsenite with solid arsenious oxide when applied to the dorsal cuticle of female ticks in the same way as with sodium arsenite, i.e. held on with rubber solution.

TABLE 5. *Effect on nymphal ticks of wool dipped in sodium arsenite and wool dipped in arsenious oxide*

Arsenite 3.7 mg./g. of wool	Arsenious oxide 3.8 mg./g. of wool
6 hr.	6 hr.
24 alive 1 moribund 1 dead	26 alive
24 hr.	24 hr.
5 alive 5 moribund 16 dead	20 alive 6 moribund
48 hr.	48 hr.
26 dead	2 alive 14 moribund 10 dead

4. THE MODE OF ACTION OF PHENOLS

(i) *The action of a phenolic fraction, boiling range 220–250° C.*

Field tests were made using a low-temperature tar acid of boiling range 220–250° C., referred to as

no. 3 fraction (Heath, unpublished). This phenol gave an initial kill of ticks, but little lasting protection. The higher phenols have bactericidal properties, and it is worth analysing the causes of the ineffectiveness of such phenols as tick dips.

Phenol no. 3 is slightly soluble in water (about 1 part in 750), and at a dip concentration of 0.5 % most of it is present as an emulsion of droplets. Ticks were put between layers of filter paper soaked in an emulsion of no. 3 fraction, but the results were difficult to interpret because the emulsion broke under these conditions and deposited the phenol on the filter paper so that the tick got a varying deposit of drops of pure phenol. It is better to consider the effect of a saturated solution of phenol no. 3.

Forty nymphs were put with a saturated solution of phenol no. 3 on glass-wool: after 15 min. twenty-two were paralysed and eighteen could progress, but after 30 min. all forty were paralysed. This test explains the killing of ticks immediately after dipping found by Heath. While there is liquid water in the fleece the phenol can act with this marked effectiveness, but it is different when the fleece becomes dry.

Wool was dipped in 0.5 % no. 3 emulsion, dried, and then conditioned at 100 % relative humidity for 24 hr. Two successive lots of ten unfed nymphs were confined with the wool in a tube with a wire-gauze top to allow ventilation. The first ten were all dead after 48 hr. and the second ten all alive after 48 hr. at 23° C. This was the most variable of the series, but other results were also variable. Nymphs survived if they crawled through the wool near to the air inlet.

It would appear as though the no. 3 were killing by its vapour, and if so, then the variability of the results of confining nymphs was due to the position of the wool in the tube and the formation of pockets of vapour. To test the effect of the vapour, a dipped lock was placed in a tube and the latter closed with a cork perforated to allow a piece of narrow glass tube to pass until its end was a few millimetres from the surface of the lock of wool. The end of the narrow glass tube was covered with wire gauze to prevent the ticks from coming in contact with the wool, while any tendency to migrate up the tube was prevented by a tight cotton-wool plug. Ten nymphs placed in the narrow glass tube were all dead in 24 hr.

The contact effect of a solution of no. 3 in lanoline was tried because that is how it must exist, at least in part, on the dipped wool fibre: 1 g. of wool held about 10 g. of dip at a concentration of 0.5 % no. 3 and this, on drying, would give 0.05 g. of no. 3. Now a sample of this wool contained 23 % wool fat, so that if all the phenol went into solution in the wool fat the result would be a 22 % solution of no. 3. I took a 33 % solution of no. 3 in lanoline for my contact tests, which is higher than would be expected. Two successive groups of ten unfed nymphs had their backs covered with a layer of the mixture, and no effect was observed even after 6 days at 20–22° C.

The ticks were free to roam about a large volume, and because of the small amount of mixture on their backs no high concentration of vapour could be built up. It is different when nymphs are kept near a large amount of no. 3 solution in lanoline. Using the same contrivance as before, ten nymphs were dead after 8 hr. at 20–22° C.

With the adult ticks also phenol no. 3 shows no contact effect. Ten females and ten males were suspended on pins with rubber solution and a 33 % solution in lanoline applied to the tips of the legs. All could still progress after 48 hr. at 23° C.

The effect of a mixture of no. 3 and lanoline was tested on the sheep using the box technique. Two tests were made, putting 0.02 g. of a 33 % solution of no. 3 in lanoline in each case on to the area of clipped fleece enclosed by a 1½ in. box. Five females were put on in each case immediately following the application of the phenol. In the first box four females attached and subsequently gorged in a normal way; but in the second box only one female attached and gorged, the remaining four females died without attaching. The first response of the ticks was to go to the top of the perforated box, in which position a few were overcome by the vapour, fell down on to the surface of the skin and died in the higher concentration prevailing there. Attachment only occurred after 12 hr., when one suspects that the vapour of the phenol had evaporated.

Phenol no. 3, therefore, is very toxic in aqueous solution, surprisingly harmless in solution in wool fat, but very toxic as a vapour. This agrees with the findings of Fitzgerald *et al.* (1942) that an unfractionated tar oil (b.p. 200–240° C.) exerts a strong fumigant effect on wheat weevils, but has little contact effect. The low toxicity of phenol no. 3 in wool fat is paralleled by the work of Foley & Lee (1942), who studied the action of antiseptics in various ointment bases. Phenol has an antiseptic action in watery gels, but was inactive in fatty bases.

(ii) The action of pure phenols

The phenol fraction no. 3 (b.p. 220–250° C.) is a mixture of phenols. It seemed better to examine two pure phenols, each a chemically known entity, and compare some of their properties. I chose phenol and 3-methyl-6-ethyl-phenol, the latter having a boiling-point of 220° C. and being comparable with the phenolic fraction no. 3 used in the dipping trials.

Two properties seemed important: the distribution between water and lanoline, and the rate of evaporation from thin films. The distribution between lanoline and water was determined by putting a weighed quantity of a known solution of the phenol in lanoline in a stoppered bottle, shaking it with a known volume of water and keeping the whole at 37° C. The aqueous layer was then titrated and the concentration in the lanoline layer found by difference. This overcame the impossibility of esti-

inating phenol in the lanoline layer directly because the latter takes up droplets of water and forms a stable emulsion.

Table 6 shows that the addition of alkyl groups to the benzene nucleus alters the distribution in favour of the lanoline.

TABLE 6. *Distribution of phenol and 3-methyl-6-ethyl-phenol between lanoline and water*

	Phenol		3-methyl-6-ethyl-phenol	
Conc. in water	0.27 %	1 %	0.06 %	0.0126 %
Conc. in lanoline	2.5 %	7.7 %	9.7 %	2.19 %
Distribution coefficient	9.3	7.7	162	175

Table 7 shows that the phenols evaporate both at 37 and 23° C. After 24 hr. a steady state is reached with phenol left in the film to the amount of only a 1-3 % solution. This loss by evaporation is in marked contrast to films of DDT in lanoline exposed under comparable conditions.

To correlate the physical and biological findings more closely ticks were immersed in various media so that loss by evaporation was eliminated. The nymphs have a spiracular system which complicates the results, so larvae being without trachea were used instead.

The larvae were immersed in the aqueous solutions at 37° C. contained in small dishes, and the lanoline solutions were put on cavity slides. It was

TABLE 7. *The evaporation of phenols from thin films*
Weight of film in mg./sq.cm. at times stated

	0 hr.	1½ hr.	4 hr.	7 hr.	24 hr.	
37° C.: 9.1 % 3-methyl-6-ethyl-phenol	1.33	1.27	1.24	1.23	1.22	=91.7 % of original wt.
9.1 % phenol	1.42	1.34	1.32	1.32	1.32	=92.9 % of original wt.
23° C.: 9.1 % methyl-ethyl-phenol	1.38	1.37	1.32	1.30	1.28	=92.8 % of original wt.
9.1 % phenol	1.60	1.53	1.52	1.51	1.50	=93.8 % of original wt.

TABLE 8. *Effect of solutions of phenol and methyl-ethyl-phenol on larval ticks at 37° C.*

	30 min.	
1 % phenol in lanoline	23 can progress 17 paralysed	
1 % 3-methyl-6-ethyl-phenol in lanoline	19 can progress 21 paralysed	
	20 min.	30 min.
Phenol 1/800 in water	20 can progress 8 paralysed	8 can progress 20 paralysed
Methyl-ethyl-phenol 1/16,000 in water	16 can progress 8 paralysed	6 can progress 18 paralysed
	5 min.	
Methyl-ethyl-phenol 1/800 in water	3 can progress 37 paralysed	

The second property is the rate of evaporation of the phenols from thin films, which occurs both from the surface of the wool (as where no. 3 in lanoline was applied to the sheep's skin) and from the surface of an attached tick.

Lanoline solutions containing 33 % of phenol no. 3 were exposed on glass slips as films varying in weight from 0.72 to 0.33 mg./sq.cm. At 37° C. there was a definite loss in 30 min., a 50 % loss in 6 hr., and almost complete evaporation in 24 hr. A slower but definite loss was found at 23° C.

The evaporation of pure phenols was also compared using a more sensitive method than the above. The phenol solutions were spread on thin sheets of mica about 12 sq.cm. in area furnished with loops of wire to allow suspension from the arm of a torsion balance sensitive to 0.02 mg.

difficult to separate the larvae from the lanoline to judge their condition at the end of the experiment. The best that could be done was to wash them in castor oil and dry on filter paper.

Table 8 shows that methyl-ethyl-phenol exerts a comparable effect in aqueous solution at about one-twentieth that of phenol. The solutions in lanoline are about the same, while equal concentrations in water show the higher phenol to be more rapid in action. The distribution coefficients lanoline-water were between 7 and 9 for phenol and 160 and 175 for methyl-ethyl-phenol which differ by a factor of 20 or so. The above tests suggest that the main difference is a lowered solubility in water rather than a higher solubility in lanoline.

Larvae were covered with a film of the phenol in lanoline solution by putting them in contact with a

film on a glass microscope slide. They were then transferred to a ventilated container at 37° C. and allowed to walk about.

	1 hr.	3 hr.
1 % phenol	18 can walk 2 affected	20 can progress 0 affected
1 % methyl-ethyl-phenol	17 can walk 3 affected	18 can progress 2 affected

No permanent effect was produced by phenol solution. With the methyl-ethyl-phenol effects were found only in the first hour and suggest that the methyl-ethyl-phenol had evaporated in the later stages of the experiment.

The reasons for the ineffectiveness of the phenols are apparent. The fact that they act in low concentrations in water is offset by their greater solubility in lanoline, but a further factor operates when these solutions in lanoline are applied to the tick, namely, the rate of evaporation from thin films. Only for a short time is the tick covered with a film of lanoline solution at the initial concentration; later in the experiment it will be exposed to a concentration reduced by evaporation.

5. THE ACTION OF DDT

(2, 2 bis-p-CHLORPHENYL 1, 1, 1, TRICHLOR-ETHANE)

(i) Site of entry of DDT

Thirteen adult females and ten males were attached to pins, and a drop of lanoline saturated with DDT was applied to the back of the tick (alloscutum). The times for developing the uncoordination of the movements characteristic of DDT intoxication (henceforward called 'drunk') were as follows (at 23° C.):

♀'s: 4 in 2 days; 4 in 3 days; 1 in 4 days;
3 in 5 days; 1 in 9 days.
♂'s: 4 in 3 days; 6 in 4 days.

Thus the posterior part of the back is only slowly penetrated by DDT. In contrast to this is the rapid effect when DDT is applied to the feet.

Ticks were so suspended that the tips of their feet just touched a film of pure DDT on a glass microscope slide. The times for becoming drunk were as follows (female ticks):

23° C. 2 at 6 hr.; 1 at 10 hr.
30° C. 3 at 8 hr.
37° C. 2 at 6 hr.; 1 at 8 hr.

Using a film of saturated solution of DDT in lanoline the times for females were:

23° C. 1 at 7 hr.; 3 at 8 hr.; 2 at 12 hr.
30° C. 4 at 6 hr.; 2 at 8 hr.

Examination of the ticks failed to show spreading of the DDT crystals or the lanoline solution to other parts of the tick's body, but the possibility is not

excluded. Five males and five females were attached to pins and the legs were drawn back out of all possible contact with the body by means of a band of 'tacky' rubber solution. DDT solution in lanoline was then applied to the tips of the tarsi. Of the males, two were drunk and three moribund after 24 hr., and of the females, two were drunk and three moribund after 24 hr. 'Moribund' designates a state at which only very small leg movements were made. With this technique only one observation is possible on any given tick, for although the solidified rubber solution can be peeled off without injury, the rest of the body is liable to be touched with DDT in the process.

Observation suggests that the delicate empodia at the tips of the tarsi are the most permeable part, and this is indicated by the following. Five females and five males were attached to pins and the end of the last joint of the legs was covered with cellulose paint. This was allowed to dry and then DDT was put over the whole of the rest of the leg. The results for the males were striking. Only one of the five was drunk in 24 hr. and the other four could all progress. A further two were drunk in 48 hr.; a fourth was drunk in 72 hr., but one could still progress at that time. This is much slower than when the tarsi are touched with DDT. The result with the females is less marked; three out of five could still progress after 24 hr., but all were much affected after 48 hr.

The conclusion from the above is that the tips of the legs and probably the empodia are the most important region of the tick for absorption of DDT.

(ii) Effect of media in which DDT has different solubilities

Unfed larvae of *Ixodes ricinus* were used, of the same age, i.e. 2 months after hatching. Groups were immersed in small glass containers of the oil solutions. After exposure the larvae were dried on filter paper and their response noted. DDT is soluble in P31 oil to about 3% at room temperatures, and soluble to about 10% in sesame oil.

The effect on larval ticks of 1% DDT solutions in P31 and sesame oils is shown in Table 9. A 1% solution in P31 acts faster than 1% DDT in sesame oil. I attribute this to the greater solubility in sesame oil and, if that is true, then saturated solutions of DDT in the two oils should have the same effect. The results in Table 9 (c) and (d) show that this is the case.

A comparison was made between 1% solutions in lanoline and 1% solutions in vaseline (Table 10). As far as possible the larvae were completely immersed in these solutions, but examination of the larvae was difficult. It was necessary to wash them in P31 oil to remove the viscous solvents, but this took time and the larvae could not be returned to the solutions.

The solubility of DDT in lanoline is about 15%,

that in vaseline is certainly lower, less than 5 %, but the high viscosity of the two media makes even an approximate determination difficult.

Once again DDT is more active at 1 % in the medium in which it is less soluble. It seems unfortunate that DDT is so soluble in lanoline, for if it were less soluble it is possible that smaller concentrations would be needed to give good protection to the sheep.

DDT is markedly different from the phenols when a 10 % solution in lanoline is exposed as a thin film on a mica slip at 37° C. No loss of weight was

Rotenone penetrates the feet of the female tick more rapidly than the dorsal surface, for the difference in rate of action is hours in the former opposed to days in the latter case.

(ii) *Effect of different solvents*

The effect of using solvents for rotenone in which the solubility varies is shown by Table 11 for unfed larvae at 23° C. immersed in the solutions in cavity slides. Both olive oil and castor oil are by themselves without effect on larvae after an immersion period of 24 hr.

TABLE 9. *Effect on larval ticks of solutions of DDT in P31 and sesame oils at 23° C.*

	Test (i) 6 hr.	Test (ii) 6 hr.
(a) 1 % in P31	0 can progress 20 drunk 1 moribund	1 can progress 65 drunk 13 moribund
(b) 1 % in sesame oil	21 can progress 3 drunk	65 can progress 7 drunk
	$\frac{3}{4}$ hr.	$\frac{3}{4}$ hr.
(c) Saturated DDT in P31	19 can progress 23 drunk	16 can progress 21 drunk
(d) Saturated DDT in sesame oil	20 can progress 20 drunk	18 can progress 24 drunk

TABLE 10. *Effect on larval ticks of solutions of DDT in lanoline and vaseline at 23° C.*

	Test (i) 5 hr.	Test (ii) 6 hr.
1 % DDT in lanoline	35 can progress 2 drunk 4 moribund	15 can progress 13 drunk 1 moribund
1 % DDT in vaseline	18 can progress 16 drunk 2 moribund	2 can just progress 20 drunk 3 moribund
	$\frac{3}{4}$ hr.	1 hr.
Saturated vaseline	16 can progress 14 drunk 6 moribund	4 can progress 17 drunk
Saturated lanoline	13 can progress 13 drunk 12 moribund	6 can progress 18 drunk

detectable after 14 days, i.e., DDT does not evaporate appreciably under these conditions.

6. MODE OF ACTION OF ROTENONE

(1) *Site of entry*

Ten adult female ticks were suspended on the ends of pins by means of rubber solution.

In five pure rotenone was put on the dorsal surface (alloscutum). Intoxication occurred as follows: one in 4 days, four in 5 days.

In the other five the feet only were brought in contact with a film of pure rotenone on a glass slide. Two were intoxicated in 2 hr, two in 4 hr. and one in 6 hr.

The difference is best seen at the end of 6 hr. when all the larvae in the olive-oil solution (a) are intoxicated, but twenty out of the fifty in the castor oil solution are (b) still capable of progressing.

This difference can be attributed to the greater solubility in castor oil (5-10 times; data of Jones & Love, 1937). If so, then saturated solutions should be much the same. Table 11 (c) and (d) suggest that this is the case. In the saturated solutions the potential is the same, but in the 0.1 % olive-oil solutions the potential is markedly higher than in the 0.1 % castor oil.

What is interpreted as a similar effect was found by Robinson (1942a). He compared 1.5 % rotenone solutions dissolved, (1) in xylene (i.e. dimethyl

phenol) 5 parts and ground-nut oil 15 parts; and (2) in xylene 5 parts, ground-nut oil 3 parts and P31 12 parts, as sprays against the tick, *Ornithodoros moubata* Murray. At a given amount of application, solution (2) was the more toxic. Robinson observed that complete replacement of the ground-nut oil by P31 in the second solution caused precipitation of the rotenone. Thus I suspect that replacement to the extent of 12 parts out of 15 would cause a marked decrease in solubility. I attribute the greater toxicity to a lower solubility of rotenone in the second mixture, which makes Robinson's data comparable with mine. Immersion of *Ixodes ricinus* larvae in Robinson's mixtures is not practicable because the large amount of xylene would alone be rapidly toxic.

Both Wigglesworth (1942) and Robinson (1942*b*) have shown a similar effect with solutions of pyrethrum in mineral and vegetable oils. Wigglesworth put 2% solutions in the different oils into cells sealed on to the backs of fifth stage *Rhodnius* nymphs.

vulnerable part of the tick's body to all the toxic substances tested. The exact point of entry of insecticides is often not considered, yet it is worth finding this if a clear understanding of the action is sought.

The second concerns the effect of the medium in which the toxic substance is dissolved and the need for knowing not only the concentration but also the solubility of the substance. In spite of the diversity of the compounds tested (arsenious oxide, phenols, rotenone, DDT) increasing the solubility at a given concentration slows the rate of action of each. Ferguson (1939) distinguishes between chemical and physical toxic agents. He suggests that solubility effects are important with the physical type, but that concentration alone will be important with the chemical type. He instances ammonia and hydrocyanic acid among the chemical type in his discussion of fumigants. One would expect these substances to behave in the same way as arsenious oxide and penetrate more rapidly from solutions in which they are

TABLE II. Effect on larval ticks of solutions of rotenone in olive and castor oils at 23°C.

	2 hr.	4 hr.	5½ hr.	6½ hr.
(a) 0.1% rotenone in olive oil (solubility 0.2%)	All can progress —	29 can progress 32 drunk	10 can progress 51 drunk	0 can progress 61 drunk
(b) 0.1% in castor oil (solubility 1-2%)	All can progress —	33 can progress 18 drunk	29 can progress 22 drunk	21 can progress 30 drunk
	2 hr.	4 hr.		
(c) Saturated olive oil	10 can progress 28 drunk	0 can progress 38 drunk		
(d) Saturated castor oil	17 can progress 30 drunk	2 can progress 45 drunk		

The average time needed to cause paralysis was about 12 hr. with 2% pyrethrum in P31 paraffin oil, while with 2% in olive oil half the insects were unaffected after 4 days. The same slowness of action was found with sesame and castor oils. Robinson (1942*b*) immersed larvae of *Ornithodoros* in pyrethrum solutions in oil and noted the time for stimulation which is the first sign of poisoning. The mean time for stimulation using a 0.022% solution of pyrethrum in P31 was 3.85 ± 0.391 min., while the mean time for stimulation for a 0.044% (i.e. more concentrated) solution in castor, linseed or cotton oil was in all cases more than 10-12 min.

There are no published data for the solubilities of the pyrethrins in different oils; but from Wigglesworth's and Robinson's results one would expect pyrethrum to be more soluble in vegetable than in mineral oils.

DISCUSSION

Two matters are worthy of emphasis in our examination of the effects of different toxic substances on the sheep tick.

The first is the discovery that the feet are the most

present as free base and acid respectively rather than salts, whether we consider this to be due to ionization or solubility. It is probable that at a given concentration increase in solubility will lower the rate of action of any substance to some extent.

If degree of saturation were all important, then all saturated solutions of a substance should be equally potent and this should apply even in media where the substance is virtually insoluble. Obviously there is a limit to this, for insoluble substances are without effect; evidently some factor has been overlooked. If degree of saturation is analogous to electric potential, then the missing factor is analogous to capacity.

The addition of a small amount of a substance to a medium in which it is slightly soluble produces a high degree of saturation; but the removal of the same small amount will greatly reduce the saturation. Conversely, with a medium in which the substance is very soluble the capacity factor is large, and small additions of substance cause small increases of potential, but, at the same time, small losses cause only small falls in potential.

In my experiments the tick is in contact with a

large amount of medium which is continually stirred by the tick's movements. As soon as a portion of liquid is depleted more is available by mixing. Any passage of the solute into the organism by diffusion causes lowering of the potential of the solution. The potential will fall slightly in a saturated solution which contains much of the substance, but markedly in a saturated solution which contains only a little. In the latter the liquid round the tick must be continually renewed to prevent the potential falling. The situation is similar to that referred to by Carter (1931) when comparing aquatic and aerial respiration. A gill in contact with water saturated with air is as efficient a means of getting oxygen into the body of the animal as a lung in contact with air; but aerial respiration has the advantage that the lung is in contact with a medium where the total amount of oxygen is large, and so there is less danger of a fall in oxygen potential.

The conclusion is that with a high solubility, although the initial potential of a saturated solution is the same as with a low solubility, yet the power to maintain that potential against the loss due to diffusion is greater. For practical purposes one needs a compromise: solvents in which the toxic body is not so soluble that a wasteful amount is needed to produce saturation nor so insoluble that depletion takes place and the organism becomes surrounded with a region virtually free of the agent.

The ideas in the preceding paragraphs are put forward tentatively. There do not appear to exist chemical data for the rate of passage of a solute from one phase into another. Such data would be relevant to our discussion of the rate of passage of a substance from the toxic solution into the tick's body, regarding the latter as a distinct phase. Physical chemists have dealt more with the final state of equilibrium and less with the processes leading to it.

We have dealt with the action of the toxic substances in the form in which they exist outside the tick; and the tick has been used as an indicator of the properties of these solutions. Our data throw no light on the action of the poisons within the tick's body, yet it is essential to understand the behaviour of the poison outside the tick's body before inferring what happens inside the tick's body. 1% DDT in P31 oil is more rapid in action than 1% DDT in sesame oil. Solubility alone seems to account for this difference and we could have predicted it without doing any experiments on ticks. Neither oil

penetrates the cuticle of the tick readily, so that we cannot attribute the difference to a superior carrier activity of the P31. Where the solvent penetrates the cuticle, as Wigglesworth (1942) finds for the bug *Rhodnius prolixus* using P31 and oleic acid mixtures, a visible mechanism is at work hastening the absorption of any poison dissolved in the oil. Wigglesworth also noted that treatment of the bug cuticle with petroleum ether increased the rate at which pyrethrum in oil was subsequently absorbed. Hurst (1943) finds that preliminary immersion of a blow-fly larva in kerosene allows alcohol subsequently to penetrate more rapidly. These situations are more complex and differences due to solubility will be added to by other factors.

The situation will also be more complex when the solvent by itself has a harmful effect. Imagine two oils equally injurious to the tick: in oil 'A' DDT is readily soluble and in oil 'B' DDT is slightly soluble. The effect of adding DDT at a given concentration to oil 'B' will produce a more toxic solution than the same addition of DDT to oil 'A'. With oil 'B' we might speak of a 'synergistic action' of the oil and DDT as the term is used by Bliss (1939). This would be another way of stating the same effect that has been described in terms of chemical potentials. It would not, as Bliss at one stage of his argument implies, give us ground for speculations on how the two drugs act at their 'site of action' in the organism. In analysing how two substances act when applied together, we must consider the possibility of one of them affecting the solubility of the other. We must know what properties are inherent in the solution itself before speculating on what occurs within the organism.

In addition to assistance already referred to in the text, I wish to thank Dr V. B. Wigglesworth, F.R.S., for many useful suggestions during the course of the work and for helpful criticism of the manuscript; and Prof. P. A. Buxton, F.R.S., under whose direction the work was begun and in whose Department it has been carried out. I owe suggestions too numerous to detail from colleagues at the School of Hygiene and Tropical Medicine and the Agricultural Research Council, but would like to mention Dr A. Milne and Dr J. R. Busvine, who have read the manuscript; and Mr G. B. S. Heath and Dr J. G. Mitchell, with whom I have worked in field trials.

REFERENCES

- ALLEN (1925). *Commercial Organic Analysis*, 3. London: J. and A. Churchill.
- BERKELEY, Earl of & HARTLEY, E. G. J. (1906). Osmotic pressures of some concentrated aqueous solutions. *Philos. Trans.* **206**, 481.
- BLISS, C. I. (1939). The toxicity of poisons applied jointly. *Ann. appl. Biol.* **26**, 585.
- BRUNNICH, J. C. & SMITH, F. (1914). Factors influencing efficacy and deterioration of cattle dipping fluids. *Qd. agric. J.* **2**, 81.

- CARTER, G. S. (1931). Aquatic and aerial respiration in animals. *Biol. Rev.* **6**, 1.
- COOPER, W. F. & LAWS, H. E. (1915). Some observations on the theory and practice of dipping. *Parasitology*, **8**, 190.
- DAVIES, W. M. & HOBSON, R. P. (1935). Sheep blowfly investigations. I. The relationship of humidity to blow-fly attack. *Ann. appl. Biol.* **22**, 279.
- DOWNING, W. (1936). The life history of *Psoroptes communis* var. *ovis* with particular reference to latent or suppressed scab. *J. comp. Path.* **49**, 163.
- ENTOMOLOGICAL INVESTIGATIONS, 1940-1. 15th Rep. *coun. sci. industr. Res. Aust.* pp. 17-27.
- 1941-2. 16th Rep. *coun. sci. industr. Res. Aust.* pp. 14-21.
- FERGUSON, J. (1939). The use of chemical potentials as indices of toxicity. *Proc. roy. Soc. B*, **127**, 387.
- FITZGERALD, J. S., RATCLIFFE, F. N. & GAY, F. J. (1942). The use of mineral oils and tar oils for wheat weevil control. *J. coun. sci. industr. Res. Aust.* **1**, 59.
- FOLEY, E. & LEE, C. O. (1942). A study of antiseptics in various ointment bases. *J. Amer. pharm. Ass.* **31**, 105.
- GREEN, H. H. and DIJKMAN, C. D. (1918). Some experiments on the fate of arsenic in the animal body. 7th and 8th Rep. *vet. Res. S. Afr.* p. 689.
- HEATH, G. B. S. Unpublished data.
- HEATH, G. B. S. & MITCHELL, J. G. Unpublished data.
- HOBSON, R. P. (1936). Sheep blowfly investigations. 4. On the chemistry of the fleece with reference to the susceptibility of sheep to blow-fly attack. *Ann. appl. Biol.* **23**, 852.
- HOSKINS, W. M. (1932). Toxicity and permeability. I. Toxicity of acid and basic solutions of sodium arsenite to mosquito pupae. *J. econ. Ent.* **25**, 1212.
- HURST, H. (1943). Principles of insecticidal action as a guide to drug reactivity-phase distribution relationships. *Trans. Faraday Soc.* **39**, 390.
- JONES, H. A. & LOVE, S. (1937). The solubility of rotenone. 2. Data for certain additional solvents. *J. Amer. chem. Soc.* **59**, 2694.
- LANDOLT-BORNSTEIN (1927). *Tabellen*.
- MACLEOD, J. (1941). Recent work on the sheep tick and its bearing on control measures. *Ann. appl. Biol.* **28**, 296.
- MILNE, A. (1945). The control of the sheep tick (*Ixodes ricinus* L.) by treatment of farm stock. *Ann. appl. Biol.* (in the Press).
- O'KANE, W. C. & GLOVER, L. C. (1935). Studies of contact insecticides. 10. Penetration of arsenic into insects. *Bull. N.H. agric. exp. Sta.* **63**, 1.
- (1936). Studies of contact insecticides. 11. Further determinations of the penetration of arsenic into insects. *Bull. N.H. agric. exp. Sta.* **65**, 1.
- RANSOM, B. H. & GRAYBILL, H. W. (1912). Investigations relative to arsenical dips as remedies for cattle ticks. *Bull. U.S. Bur. anim. Ind.* **144**, 1.
- RICHARDSON, C. H. & SHEPARD, H. H. (1930). The effect of H ion concentration on the toxicity of nicotine, pyridine and methylpyrrolidine to mosquito larvae. *J. agric. Res.* **41**, 337.
- ROBINSON, G. G. (1942a). The relative toxicity of rotenone and pyrethrum in oil to the Argasid tick *Ornithodoros moubata* Murray. *Bull. ent. Res.* **33**, 273.
- (1942b). The penetration of pyrethrum through the cuticle of the tick *Ornithodoros moubata* Murray (Argasidae). *Parasitology*, **34**, 113.
- TATTERSFIELD, F. & ROBERTS, A. W. R. (1920). The influence of chemical constitution on the toxicity of organic compounds to wireworms. *J. agric. Sci.* **10**, 199.
- WATKINS-PITCHFORD, H. (1911). *An illustrated pamphlet on tick destruction and the eradication of east-coast fever and other South African diseases by dipping*. Natal: P. Davis & Sons.
- WIGGLESWORTH, V. B. (1942). Some notes on the integument of insects in relation to the entry of contact insecticides. *Bull. ent. Res.* **33**, 205.
- WOOD, J. K. (1908). Amphoteric metallic hydroxides. Part 1. *J. chem. Soc.* **93**, 411.
- WOODWORTH, C. E. (1938). The reactions of wireworms to arsenicals. *J. agric. Res.* **57**, 229.

(Received 10 May 1945)

Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 8 December 1944, at 11.30 a.m. in the Imperial College of Science and Technology; the President, Prof. W. Brown, F.R.S., in the Chair.

Morning Session.

Bloat or eelworm disease of onions: recent investigations. By T. GOODEY, D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans, Herts.

Afternoon Session. *Symposium on the carrot fly.*

1. The biology and control of the carrot fly. By F. R. PETHERBRIDGE, M.A., D. W. WRIGHT, M.A. and D. G. ASHBY, B.A., School of Agriculture, Cambridge.
2. The carrot fly in the Midlands. By A. ROEBUCK, Midland Agricultural College, Loughborough.
3. Investigations on the control of carrot fly (*Psila rosae* F.) in gardens. By G. Fox WILSON, R.H.S. Laboratories, Wisley, Surrey.

Bloat or eelworm disease of onions: recent investigations

By T. GOODEY, D.Sc., *Institute of Agricultural Parasitology, St Albans*

After some introductory remarks dealing with the history of onion bloat, its first description by Beijerinck in 1883 and the subsequent early investigations of Ritzema Bos which settled the identity of the parasite and established the main symptoms of attack, the author dealt with researches which he has carried out during the past 2 years (Goodey, 1943, 1945 *a, b*; see also Wallace & Wood, 1943), whereby he has established the fact that the stem eelworm, *Anguillulina dipsaci*, can occur in a viable condition on onion seed in commerce.

The present war brought about a cessation of the importation of onions into Britain. There was, as a consequence, a great increase in onion growing in this country and, concomitantly, a considerable increase in the incidence of onion bloat. This led the author to the view that it might be well to attempt to find out if the parasite was being dispersed on onion seed. Samples of onion seed supplied by Mr C. C. Brett and Mr E. G. Thompson of the National Institute of Agricultural Botany, Cambridge, were tested for the presence of the eelworm, and although the first 248 samples (mainly of foreign origin) proved negative, seven out of forty-six samples of English-grown seed proved positive in that they carried examples of the parasite in a quiescent but living condition. This result was followed by the discovery

of the parasite in the scapes and inflorescences of onions being grown for seed in material supplied by S. G. Jary, from a crop growing in Sussex, in August 1943. Later it was established that the eelworms could not be removed from the seed coats by the ordinary processes of winnowing and cleaning. The author then gave an account of experiments carried out during 1944 whereby the course taken by the eelworms from parent bulbs with a mild patent or latent infection was traced. This was illustrated with lantern slides and it was shown that the eelworms make their way up the entire length of the scape, invading the inner parenchyma in their course and multiplying freely in the tissues until they reach the inflorescence. Here they continue on their course up individual flower pedicels and into all the floral organs. As the seed capsule enlarges and the seeds begin to ripen the eelworms occur in considerable numbers in the tissues of the flower receptacle and placenta whence they can easily become attached to the seed, especially in the region of the hilum.

Finally, an account was given of a successful method for the destruction of the parasite on onion seed by fumigation with methyl bromide. The method was described, and it was shown that fumigated onion seed is not injured by the treatment but has as good a germination as untreated seed.

REFERENCES

- GOODEY, T. (1943). *Anguillulina dipsaci* in the inflorescence of onions and in samples of onion seed. *J. Helminth.* **21** (1), 22-30 (issued March 1944).
- (1945a). *Anguillulina dipsaci* on onion seed and its control by fumigation with methyl bromide. *J. Helminth.* **21** (2/3), 45-59.
- GOODEY, T. (1945b). Further observations on *Anguillulina dipsaci* infestation of the onion scape and inflorescence. *J. Helminth.* **21** (2/3), 60-8.
- WALLACE, E. R. & WOOD, J. (1943). Stem eelworm in onion bulbs, probably seed-borne in origin. *J. Helminth.* **21** (1), 33-6 (issued March 1944).

The biology and control of the carrot fly

By F. R. PETHERBRIDGE, M.A., Dip.Agric. (Cantab.), D. W. WRIGHT, M.A. and D. G. ASHBY, B.A.
School of Agriculture, Cambridge

(With 1 Text-figure)

This investigation was started in 1941 and is still in progress.

The emergence and habits of the flies were studied by means of cages and by sweeping the vegetation with nets. Observations show that attacks of the first generation of larvae while the carrots are still young can be very serious and can destroy a large number of young seedlings. In 1944 all the seedling carrots growing in small plots in a field in which the flies were very numerous were completely destroyed by this early attack.

There is a marked variation in the time of emergence of the first generation of flies (see also Barnes, *J. anim. Ecol.* **11**, 69). In the four years during which observations were carried out the following are the periods of maximum emergence from fen soil near Chatteris, Isle of Ely:

1941	30 May- 5 June
1942	15 May-21 May
1943	4 May-13 May
1944	9 May-23 May

Flies continued to emerge over a longer period from fen soil (Chatteris) than from medium loam (Cambridge) or light loam (Norfolk and Suffolk).

Far more flies emerge from carrots sown in mid-April than from those sown in mid-May and fewer still from those sown in early June.

IMPORTANCE OF SHELTER

The distribution of the flies is largely determined by the weather and the shelter afforded by vegetation. In general, the presence near a carrot field of thick shelter such as a potato crop, a bed of nettles, a dense hedge, etc., decides where most of the carrot flies will aggregate, or where the heaviest maggot attack will occur. This effect was artificially produced in an experiment done at Chatteris in 1944.

In a large carrot field at Chatteris two strips of maize 90 yd. long by 6 yd. wide were sown as shown in Fig. 1.

In November two series of carrot samples were taken, one at right angles to the maize growing towards the middle of the field (A) and the second from midway between the two maize strips and parallel to the first (B). The infestations recorded were as follows:

Yards from line drawn through edge of both maize plots	0	5	15	20	40	50
Transect A (mines per 100 carrots)	44	40	12	8	12	10
Transect B (mines per 100 carrots)	8	8	8	6	4	6

The shelter provided by the maize had therefore greatly increased the attack; producing, in fact, a headland pattern of infestation in midfield.

CONTROL MEASURES

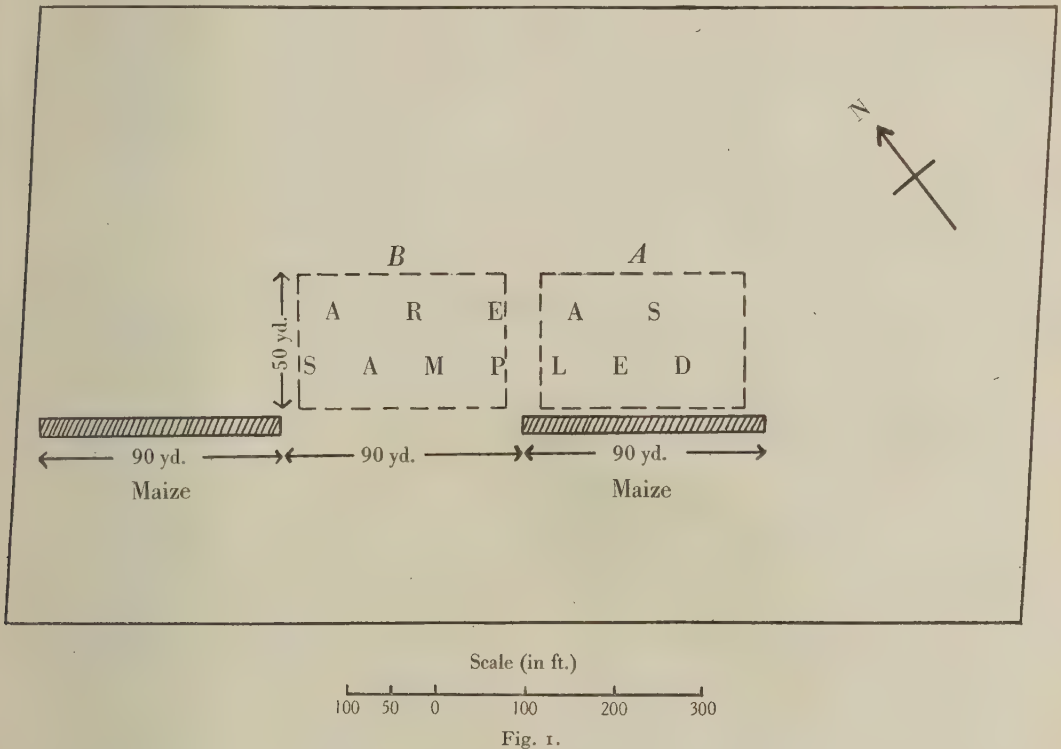
Cultural. Carrots grown in districts remote from carrot-growing areas are not likely to suffer much from carrot-fly damage for several years. Early carrots should be grown as far away as possible from maincrop carrots. Where carrot fly is troublesome maincrop carrots should not be sown until mid-May or later.

The practice of ploughing-in carrots which are badly mined should be given up as this increases the attack the following year. Badly attacked carrots should be lifted in October and disposed of. Clamping at the end of October brings about a marked reduction in the deterioration of the carrots. Large fields are preferable to small fields, as there is relatively less shelter there. Hedges, dikes, nettle beds, etc., should be kept trimmed, as with less shelter fewer flies are retained.

Chemical. A poison-bait method of controlling this pest, based on the sheltering habits of the flies, has proved an economic success. The poison-bait solution containing 0.8% sodium fluoride and 2.5% molasses is sprayed on the hedges, dikes and other places where the flies shelter. For this a mobile power-operated machine delivering the spray through coarse nozzles is used. For the first generation of flies at least six applications and for the second generation at least ten applications are necessary.

60 lb./sq.in. was used against the second generation on a 20-acre field of early May-sown roots. Fifteen applications were made and the results were fairly satisfactory as shown in the following table.

	No. of mines per 100 carrots		Unsaleable	
	Control	Treated	Control	Treated
Headland	131	27	11	0
Midfield	48	11	1	0



These are applied at 3-4 day intervals. For the second generation a strip of carrots 4 yd. wide next the hedge or dike is also sprayed by means of a boom carrying ten nozzles. This treatment has been successful in each of the four years under review. The following are the figures for 1943 and 1944:

	1943	1944
Total acreage of carrots treated	858	1190
Total acreage of carrots sampled	763	1010
Percentage of sampled acreage with 90% or more clean carrots	79	80

The cost of the materials for one spray application is as follows: for the first generation about 9d./acre and for the second generation about 1s. 6d./acre.

In 1944 a horse-drawn power-operated machine carrying three booms and giving a pressure of

A machine of this kind can be used on the farm for other spraying operations; the poison bait is easily cleaned out.

At the end of the 1944 season preliminary experiments were carried out to test the value of DDT in killing carrot flies.

Carrot foliage sprayed once with emulsions containing 0.5% and 1.0% DDT (commercial purity) respectively gave a 90% kill at both concentrations up to 5 weeks after the treatments.

In September sufficient DDT was obtained to do a small-scale field trial. Two and a half acres of carrots were sprayed on 15 Sept. with 0.5% DDT emulsion at the rate of about 50 gal./acre. After an interval of 3 weeks the DDT was still effective and nearly 100% of the carrot flies were killed.

These experiments suggest that DDT is likely to be more economical for the control of carrot fly than sodium fluoride.

They also suggest that DDT will be successful if used at long intervals, e.g. about 2 weeks, instead

of the 3-4 day interval necessary in the case of sodium fluoride. It is also probable that DDT emulsion can be used successfully in gardens and allotments where the abundant shelter prevents the poison bait method from being successful.

The carrot fly in the Midlands

By A. ROEBUCK, *Midland Agricultural College, Loughborough*

The area considered lies along the sides of the River Trent between Newark and its junction with the Humber. It can conveniently be divided into three districts. One district, in Nottinghamshire, lies east of the Trent and extends northwards from Newark nearly to Gainsborough. The soil is very light and sandy, sometimes almost pure blowing sand and sometimes darkened to a grey colour with organic matter. A second district lies east of the Trent in Lincolnshire north of Gainsborough. The area centres round Scotter Common and consists of light blowing sands which tail off northwards on the ironstone sands north of Scunthorpe. These two districts grow primarily carrots. The third district lies west of the Trent north of Gainsborough. It is a very distinct area enclosed by rivers and known as the Isle of Axholme. It is rich carr and warp land and the farmers specialize in red beet, celery, carrots and potatoes.

INTRODUCTION

For some years succeeding the last war celery and carrots were regarded as highly speculative crops and the farmers were prepared to accept them as such. With growing competition from other areas and with the advent of the period of depression there was a change of attitude.

Up to 1928 experimental work against carrot fly was largely devoted to the trials of various deterrents for use on allotments. Naphthalene had given us considerable control under such conditions. During this period farmers were always advised to sow later to avoid the first generation. Gradually the dates of sowing were made later and later until June became the popular month.

Some farmers went in the opposite direction and sowed earlier and earlier in order to recapture the bunched carrot market. This was not successful and had to be abandoned. When the crop was early enough to avoid carrot fly the carrots ran to seed and were useless.

THE ATTACK ON CELERY IN FIELDS

The first field scale trials we made were on celery in the Isle of Axholme in 1928 and 1929.

Usually it is the second generation which causes the trouble. The plants are not killed. The outer

stalks are tunnelled at the base and destroyed. As new ones appear they are also destroyed and so the plant makes no headway.

Against this attack considerable success was obtained by dusting with powdered naphthalene: four dressings of 28 lb. each per acre were applied with a dust gun on 1, 12 and 27 July and 14 Aug. A 25 % increase of crop was obtained. This represents much more financially.

The attacks appeared again in 1935 and have not been severe on celery fields since then, although there were some slight ones in 1943. In that year the first generation attacked the young plants in the planting-out beds and the fly killed the plants, as they were much smaller.

THE EARLY WORK ON CARROT FIELDS

In 1930 and 1931 carrot fields were treated with naphthalene. We dressed acre plots in fields with bad reputations for carrot fly attacks.

Powdered naphthalene was used at the rate of 1 cwt./acre applied on 26 May, 5 and 15 June. This gave good results on several fields. A 6-acre field was dressed completely with creosote salts at the same rate and approximately the same dates with good results.

Two experiences in 1931 shook our faith in the treatment. A large field (20 acres) was dressed with creosote salts. Some hot days followed. The fumes were too strong and workers were unable to stand them. Emboldened by former successes a field at Scotton was sown on 28 Apr. 1931. The season turned out to be a bad fly year. The whole field was dressed with 1 cwt. powdered naphthalene per acre on 26 May and again on 3 June. $\frac{1}{2}$ cwt. dressings were given on 7, 10, 14, 17, 21 and 28 June. In spite of this the crop was ploughed up. All neighbouring crops were ploughed up where the date of sowing had been earlier than 7 June.

In 1932 and onwards we changed to green tar oil, first used by Dr Kenneth Smith, which appeared to be equally deterrent to the fly and less harmful to workers. After many trials the best carrier found was North African phosphate: 1 % green tar oil was mixed with it, the mixing being done by machinery

by a local manure manufacturing firm. In 1934 the green tar oil was raised to 2%.

This has been a fair palliative and is still used by some growers. This strength is as much as the hand of the man broadcasting can stand. There is just a slight stinging of the skin when 2% strength is used.

Study of parasites (1936-41)

From 1936 other methods were sought. With the help of Dr Barnes the parasites were studied. Work was started on studying the movements of the flies. Larvae and puparia were collected annually in February or March.

One important point impressed me during these years—the greater ease with which one could collect larvae in the clamp bottoms. The farmers were collecting practically the whole population in a number of congested areas. The question arose, could we not devise a better control by tackling them here?

The assembling of the flies and their subsequent dispersal

With the advent of the war it was possible to detail someone to live on the spot for a time. An area was chosen where carrots had not been grown for many years, and where carrot fields were few, but where carrot fly had appeared as a pest. On this particular farm one field had been badly attacked in 1940. A field some distance away was to be cropped with carrots in 1941.

The field workers were to study the emergence of the flies on the old field, see how they got to the new field and study the oviposition. This they did quite well and the results have been published in the *Annals of Applied Biology* (1942, 29, 115-25).

Broadly speaking the flies after emergence collect in the hedges and remain more or less in or near them for a long period. Dispersal is in theory concentric, but in practice it tends to be along hedgerows.

Wind has its influence. They seek shelter from it, but after flights in the fields during the day they tend to drift down wind back to shelter rather than

face the wind to return to what may be their old shelter.

It was clear that the flies spent the night in the hedges and only made flights from them on relatively still days.

Some contact killing spray applied in the morning and evening would seem to offer considerable prospect of success against the pest at this stage.

Emergence of the first generation of flies

This is an abrupt phenomenon. Dr Barnes published curves for the six years and notes that 'the emergence curve is noteworthy because of the abruptness with which the peak of emergence is usually reached and the quickness with which the majority of flies are on the wing'.

The weekly peak is round about 14 May, but has varied from 7 to 21 May.

Oviposition

In 1941 the beginning of oviposition was missed but the period ended abruptly on 25 June. Oviposition was watched and the period of the day carefully observed. The period of maximum oviposition was from 4-8 p.m. (G.M.T.).

PRELIMINARY TRIALS OF SPRAYS

Some preliminary trials have been made using a tar distillate wash, various pyrethrum preparations, nicotine and paradichlorobenzene.

Pyrethrum gave very good results, but cannot now be used owing to the war. Paradichlorobenzene was very efficient, but killed the foliage. The others were not of any use.

We feel that a killer such as pyrethrum (or DDT) can be used with great effect when conditions become more normal. One or two sprayings should be sufficient.

We have not yet made satisfactory progress against larvae or puparia in the clamp bottoms, but feel that a combination of these two methods of attack will materially check this pest.

Investigations on the control of carrot fly (*Psila rosae* F.) in gardens

BY G. FOX WILSON, *R.H.S. Laboratories, Wisley, Surrey*

(With 7 Text-figures)

At the request of Dr H. V. Taylor, Commissioner for Horticulture, investigations were commenced at Wisley in 1942 of small-scale measures of control against carrot fly under the conditions prevailing in gardens and allotments.

Observations on the biology of *Psila rosae* have been undertaken by Baker *et al.* (1942), Petherbridge

et al. (1942) and Petherbridge & Wright (1943), and it is unnecessary to discuss further the life history of the pest, its food plants and the nature of the injury caused except in so far as they affect the investigations at Wisley. The control measures to receive special attention during 1942-4 were chiefly cultural, and included the effect of (i) staggered sowings (Apr.-

July), (ii) date of harvesting, and (iii) site. Further measures were included in 1944, namely, (iv) absence of cultivation (i.e. no singling, hoeing or weeding operations) and (v) chemical, in the form of a poison-bait mixture, to ascertain their relative effectiveness upon the incidence of attack on five varieties of carrot. These investigations were planned solely in relation to outbreaks of carrot fly in gardens, and no attempt was made to consider large-scale infestations, as these have received due consideration by Advisory Entomologists elsewhere.

Site of beds. The carrot beds were situated on the eastern and western sides of the dahlia border, with both southern and northern aspects. Each bed was 92 ft. long and 10 ft. wide, which allowed ninety rows of carrots in each bed with 1 ft. space between the rows. The beds were bounded by 8 ft. high hornbeam hedges on the northern and southern sides respectively, while some beds were bounded by similar hedges on their eastern and western sides, thus allowing the effect of micro-climatic conditions to be studied (Fig. 1).

Varieties. Two varieties, Early Market and Early Nantes, were grown in 1942, while three additional varieties, Danvers, James's Scarlet Intermediate and New Early Scarlet Horn were grown in 1943-4. Four rows in 1942 and two rows in 1943-4 of each variety were sown on each border at nine fortnightly intervals from 29 Mar. (earliest) to 22 July (latest).

Cultivation. The soil at Wisley is considered good for carrot cultivation, and consists of Bagshot Sand over gravel. The drainage is naturally good, and the ground has been cultivated for a variety of crops over a number of years. The ground was dug annually during March, and received dressings of bone meal, superphosphate, sulphate of potash and sulphate of ammonia (5:3:2:1 ratio) and leaf mould, this being the only organic manure available. A dressing of hydrated lime was raked in 6 weeks before sowing.

The germination of some of the earlier sowings was retarded owing to drought, while germination on the north beds was invariably slower than that on the south beds owing to the shade cast by the hedges and to the lower temperatures there prevailing, especially during the early part of the season.

The rate of sowing was 3 g. of seed per 10 ft. row, while 2 g. were used on the bed which was left unsingled and unweeded. The rows were singled and weeded as occasion arose to a distance of 2 in. which gave an average of sixty roots per row.

Sampling technique. While a careful study was made of the *Carrot Fly Sampling Leaflets* (dated 16 Jan. and 11 Feb. 1942) issued by the Statistical Department, Rothamsted Experimental Station, it was considered that the random sampling therein outlined for field sampling, would in the small area under carrot cultivation at Wisley result in considerable inaccuracies as to the estimated percentage of damage. The more laborious method of washing

and grading every root was therefore adopted, involving examination of several thousand roots each year (1942, 9400; 1943, 5000; 1944, 15,600). The smaller the area of the experimental plot, the greater the number of samples that require to be taken, otherwise a very low assessment of the true figure is obtained.

The roots were graded as clean (o), slightly damaged (1-2), and badly damaged (4-6). It was found that those classed in 1942 as moderately damaged (3) were as unsaleable as those in the 4-6 category, and were, therefore, classed as unsaleable.

Deterioration in attacked roots left in the ground occurs throughout November, especially in the case of the April and May sowings, and the percentage of unsaleable roots is found to rise considerably after mid-October.

(i) *Effect of sowing date.* Sowings made from the end of March to the third week in June resulted on the average in the same general intensity of attack, but progressively decreased on carrots sown from the end of June to the third week in July (Figs. 2, 4, 6).

Climatic conditions affect the degree of attack throughout the season, and especially among the earlier-sown carrots. This is due not only to the effect of weather upon the length of period over which the flies emerge, their dispersal and their movements from shelter to the beds, but also upon the state of growth of the seedlings and their relative attractiveness to the egg-laying females. An early attack which coincided with a period of drought in 1943 resulted in a very high percentage of unsaleable roots. A similar outbreak which occurred during the same period in the Lend-Lease Vegetable Trial Grounds at Wisley was, however, arrested by overhead irrigation.

The advantage of early singling when the seedlings are small ($\frac{1}{2}$ -1 in.) is that the eggs of the carrot fly are less likely to be found around plants with cotyledons or with one rough leaf (Petherbridge *et al.* 1942), though it was found that carrots are liable to attack while still in the cotyledon stage. To delay singling until later will invariably result in as severe an attack as on those carrots that are sown during the peak emergence period of the flies. To sow thinly will reduce the amount of singling to the minimum, and the method of supplying carrot seed in 'paper-tape' rolls would probably largely overcome the disadvantage of singling, but this may not be practicable with carrot seed owing to the normally low percentage of germination (c. 50%).

The operation of singling should be carried out during the earlier hours of the day to escape the fly's period of activity, and should be followed by the consolidation of the soil along the rows of seedlings by treading or by drawing the soil up to the roots in a manner of partial earthing-up. A thorough watering should then be given to consolidate further the soil

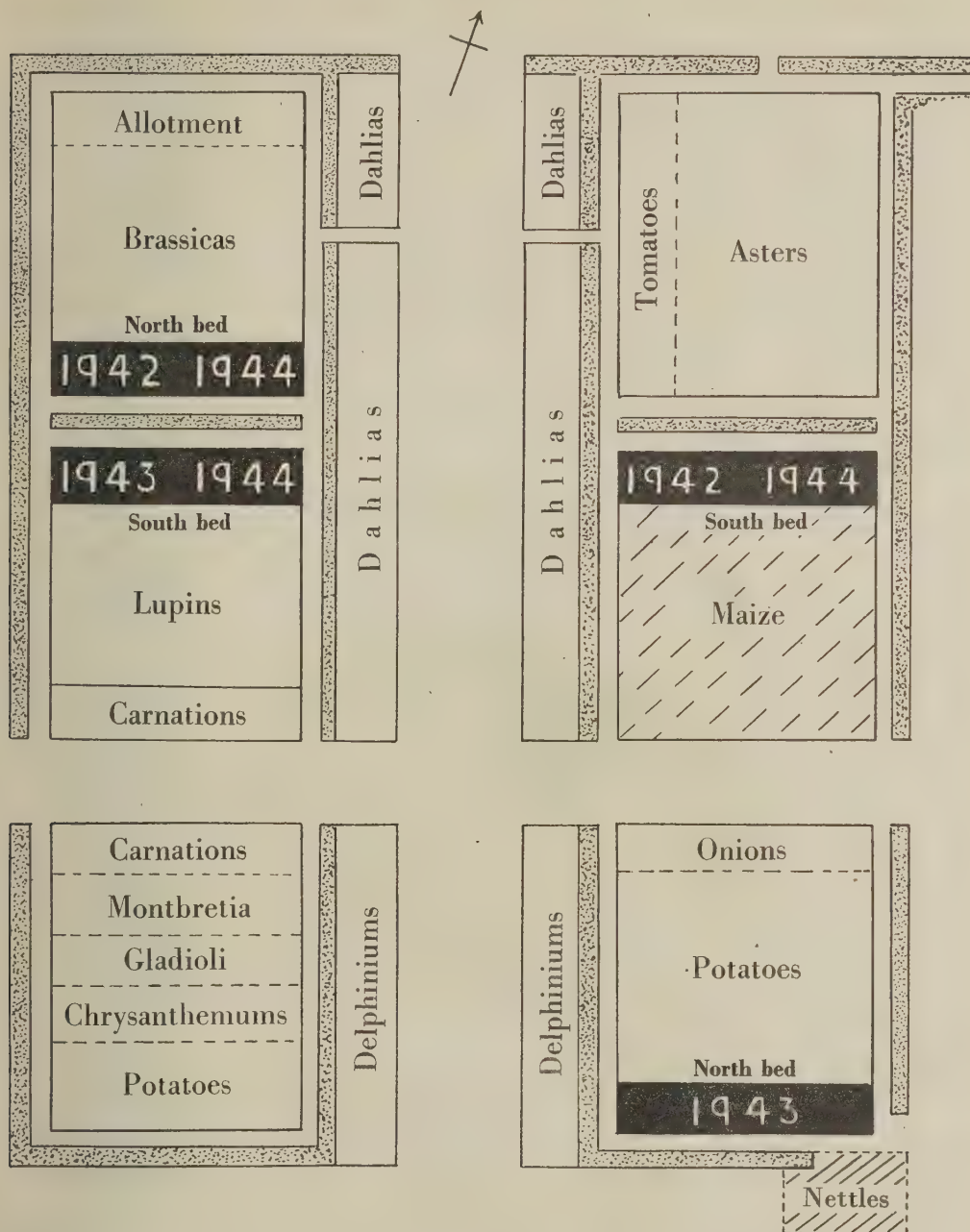


Fig. 1. Plan of Beds.

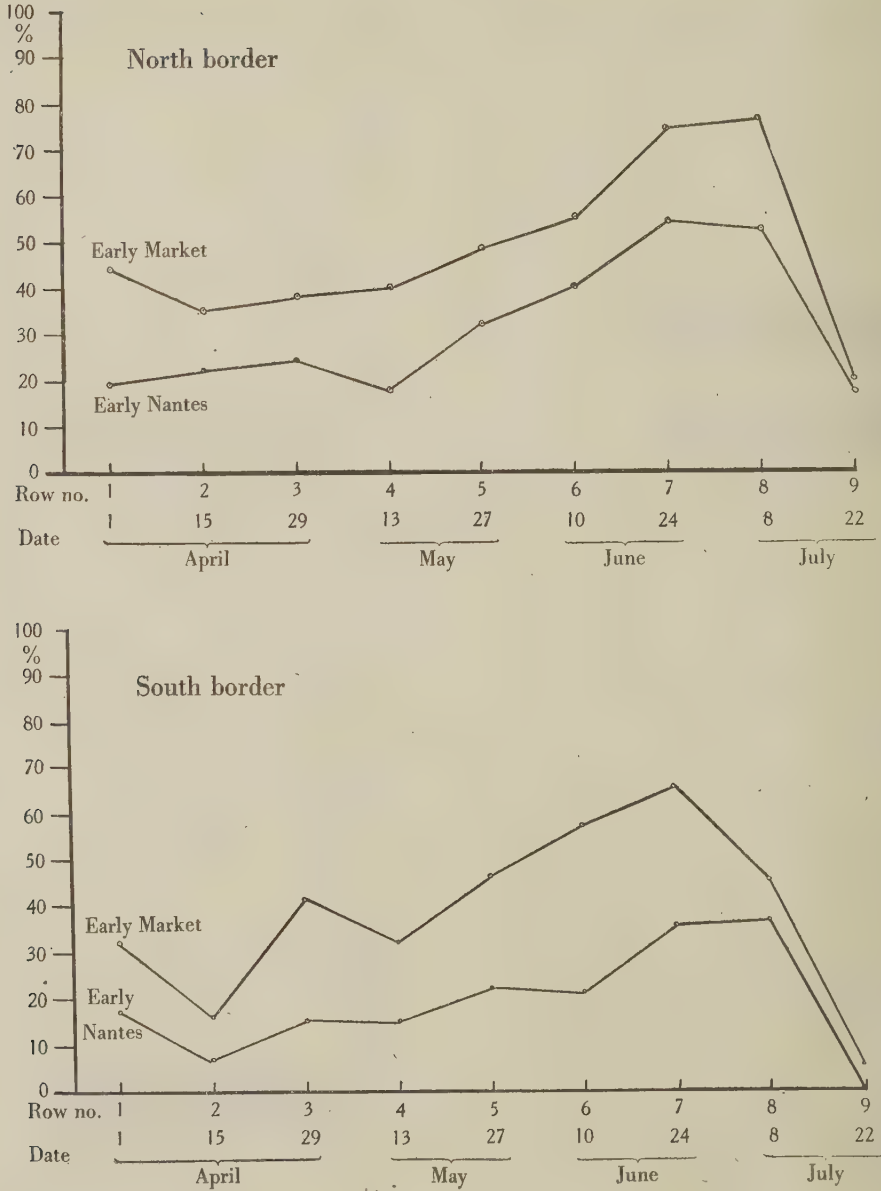


Fig. 2. Percentage of unsaleable roots.

around the singled plants. With light infestations, attacked seedlings should be lifted and burned, as the eggs are seldom distributed evenly and equally over the beds. The larvae will move along a row of seedlings in the manner of onion fly in spring-sown seedling onions owing to there being insufficient food to fulfil their requirements in a single plant (Fox Wilson, 1943).

(ii) *Effect of harvesting date.* The delay in harvesting the 1942 crop resulted in a higher percentage of unsaleable roots than if the roots had been lifted a month earlier. Early lifting (October) avoids deterioration of the roots, and harvesting after this period results in definite larval movements from root to root in the rows and a substantial increase of damage. Again, the earlier clamping of carrots is advisable not only to avoid larval movement in the standing crop, but to reduce the loss from frost injury.

All heavily infested roots should be fed to animals, and neither clamped or dug in. The vast majority of the larvae do not leave the carrots in autumn, but pupate in late winter and early spring, both in the case of roots left standing and in clamped carrots.

The weight of crop was found to be dependent upon the date of sowing and upon weather conditions. In 1942 the earliest sown (April) carrots gave the highest yields which progressively decreased as the season advanced (Fig. 3). The late April sowing, however, gave a lower yield than the mid-May sowing, due to a period of drought from which the plants never completely recovered. In 1943-4 the yields from the late May and early June sowings respectively were higher than those made during April and mid-May and, here again, climatic conditions were the factor concerned (Figs. 5, 7).

(iii) *Effect of site.* The tendency of the carrot fly to assemble in large numbers in hedges and other shelter is very marked. Coarse herbage, growing crops (especially potato) and weeds (especially nettles) provide adequate and alternative shelter against wind. There is a definite movement from hedges and other shelter to the carrot beds from 4 p.m. (G.M.T.) onwards, though again weather conditions, more especially air movement, sunlight and humidity, will affect this movement. It was clearly demonstrated that the greater population of flies occurred on the side of the hedge sheltered from wind. The prevailing wind being south-west resulted in the most severe infestations occurring each year on the beds facing north where protection was afforded by hedges on the southern and western sides (Fig. 1). In the case of beds with a southern aspect, the highest percentage of unsaleable roots, even in those rows where the general attack was high, was lifted from that part of the row nearest the hedges. This is due to the fact that the flies tend to make short trips from the hedges to the beds when the wind is blowing lightly. The heaviest infestation occurred in 1943 on roots growing in a bed with a nor-

thern aspect protected from southerly and westerly winds by hornbeam hedges and with a large bed of nettles growing at the eastern end (Fig. 1). While there was a definite daily movement of flies between the hedges and the beds, it was found that many of the flies remained on the beds among the carrot foliage during warm, calm weather, when conditions were specially favourable to egg-laying. This habit of the carrot fly in avoiding windy positions suggests that the choice of site for a bed should be in as open a position as possible and away from hedges, close fences, weed beds and rough herbage which provide natural shelter. If sowing is unavoidably near hedges, as it may well be in many gardens and allotments, a southern rather than a northern and western aspect is advisable so that the prevailing wind will tend to drive the flies to sites where there is protection from air movement.

A further disadvantage in the proximity of hedges, especially privet and elm, to the beds is the competition for water that arises between the crop and the hedge plants. The roots of such hedges are so greedy of moisture that they extend far into neighbouring beds, causing the plants to wilt and to react more severely to comparatively light attacks of fly. The scything of nettles and other coarse weeds in the neighbourhood of carrot beds is desirable, while potatoes should be grown some distance away to avoid the provision of shelter-areas.

Carrot foliage in itself does not appear to provide favourable shelter for the flies, which prefer other shelter during periods of high temperature, intense sunlight and wind.

(iv) *Effect of cultivation.* A more correct term to be applied to this series of investigations would be absence of cultivation in that an avoidance of attack may be possible by allowing the plants to grow without any disturbance from singling and weeding other than the cutting down of the coarser weeds, e.g. docks and 'fat hen'. In this way, the element of attraction in the nature of bruised carrot foliage and soil disturbance during singling and hand-weeding would be eliminated. Such weed-ridden beds, however, serve as shelter for the flies, though fewer eggs were laid among the carrots than on those in the neighbouring cultivated beds. The greater percentage of unsaleable roots lifted from the uncultivated bed was due, however, to their size rather than to attack by carrot fly (Fig. 6).

While the effect of allowing carrots to grow in a weedy, unsingled bed is that there is less attraction to the egg-laying females owing to the absence of odour from crushed foliage and from loosened soil around the roots, the disadvantages are the poor quality of the roots owing to overcrowding and to the competition that exists between crops and weeds, especially during periods of drought, so that what is gained in quantity (yield) is lost in quality (size of roots).

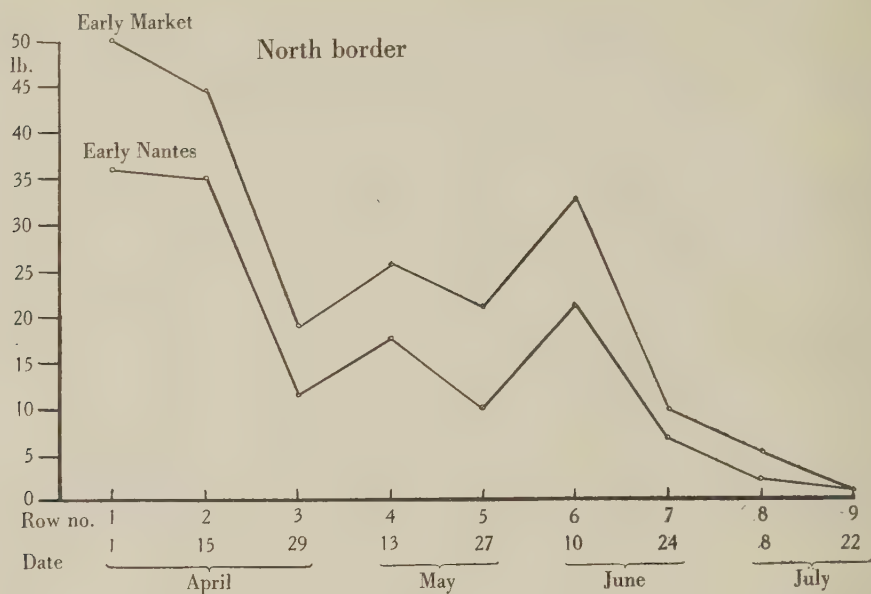


Fig. 3. Weight of roots, 1942.

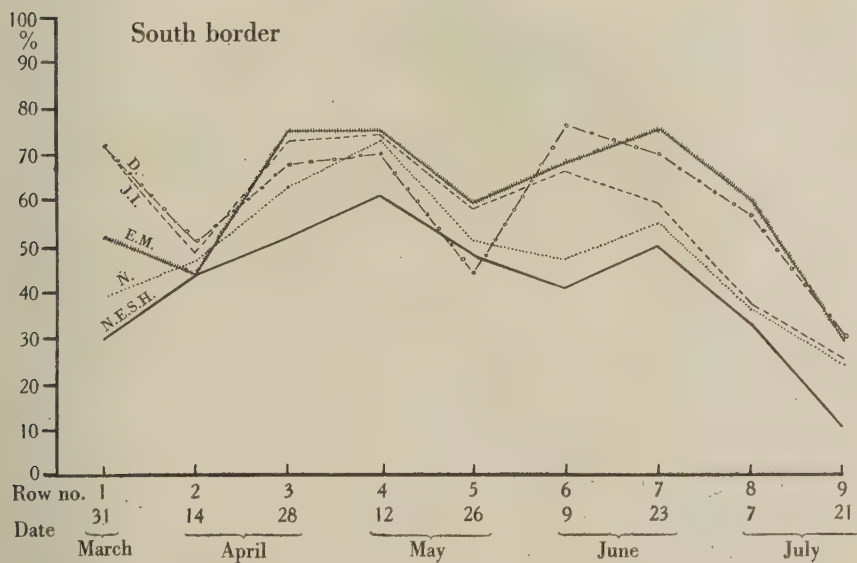
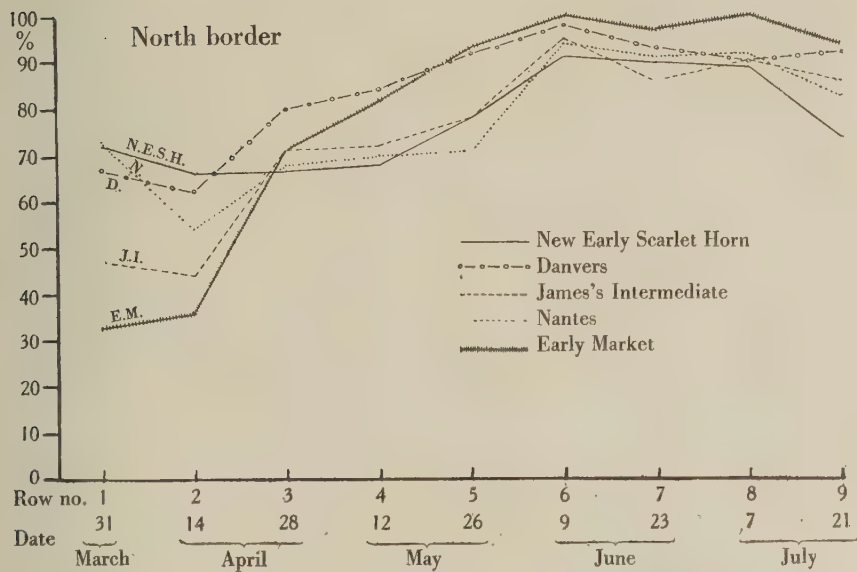


Fig. 4. Percentage of unsaleable roots, 1943.

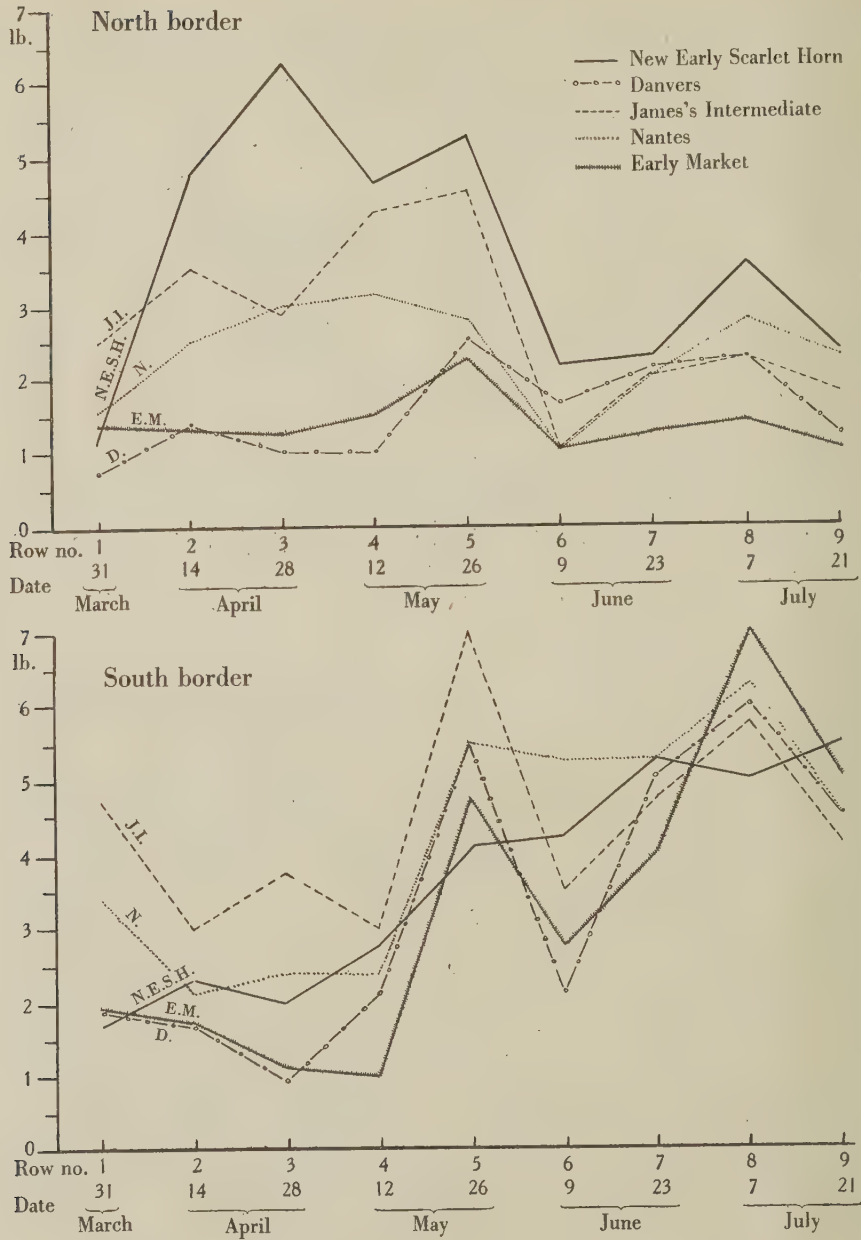


Fig. 5. Weight of crop, 1943.

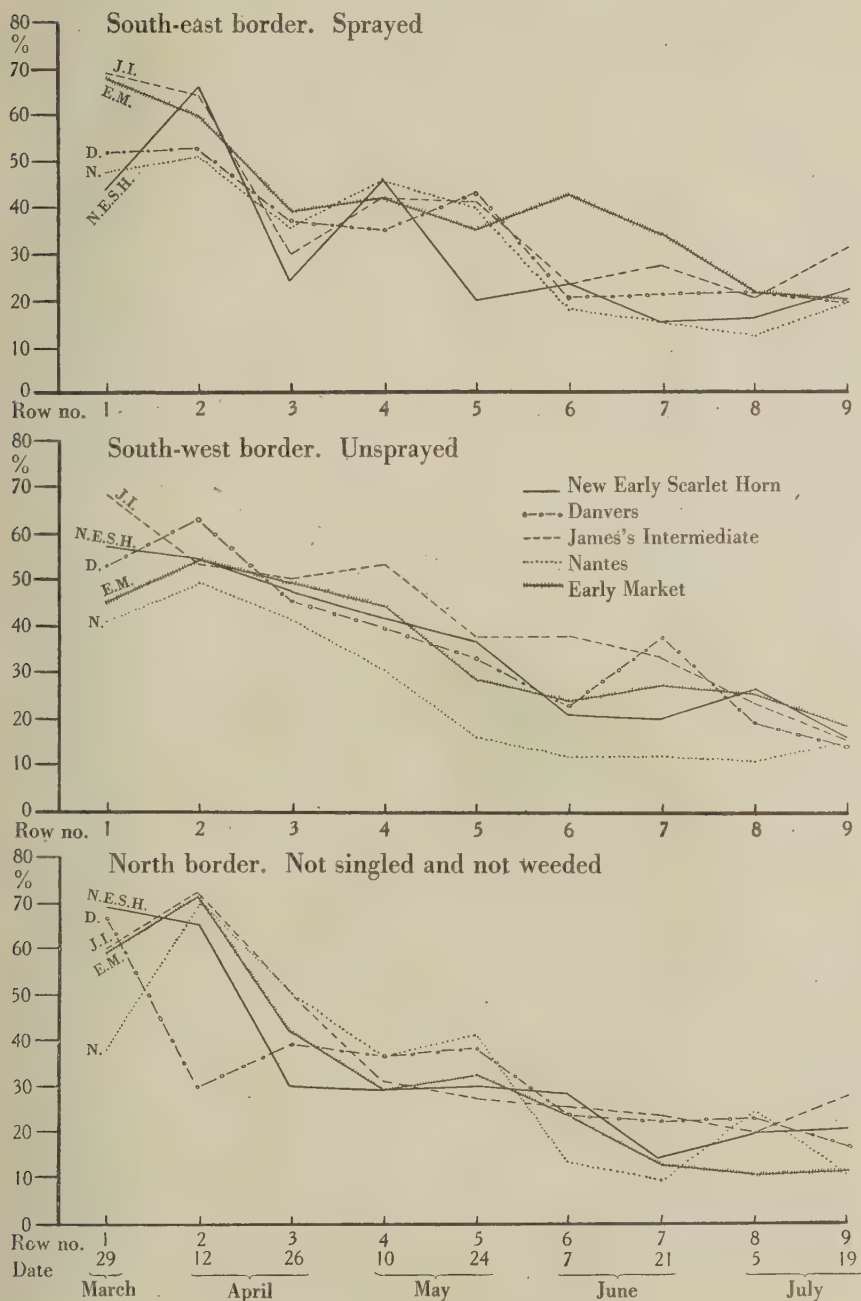


Fig. 6. Percentage of unsaleable roots, 1944.

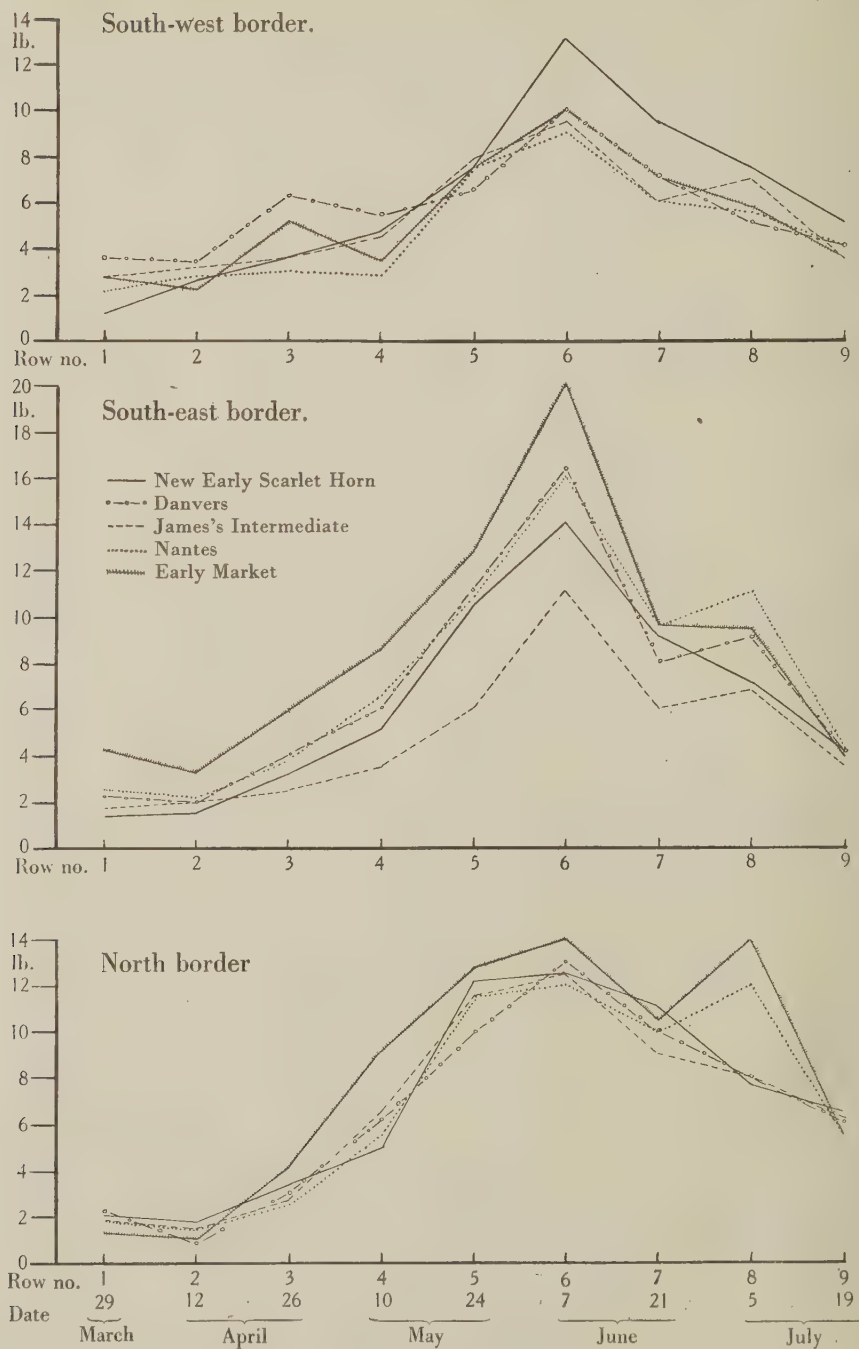


Fig. 7. Weight of crop, 1944.

The length of the taproot in those plants grown in the unweeded bed was significantly greater owing to this competition for soil moisture.

It is hoped in 1945 to conduct a further series of experiments whereby the rate of sowing is halved and the distance between the rows is lessened to enable competition to occur between carrots rather than between carrots and weeds.

(v) *Effect of poison bait.* The application of a poison-bait mixture consisting of 0.8% sodium fluoride and 2.5% cane molasses in water in successfully controlling the first generation and reducing the population of second generation carrot flies in the field has been demonstrated by Petherbridge *et al.* (1942) and Petherbridge & Wright (1943). An investigation was carried out at Wisley in 1944 to test the effect of this bait under garden conditions.

Success is dependent upon correctly timing the applications both in regard to the emergence dates of the first generation of flies and the time of day when the flies are sheltering in herbage prior to their movement from shelter to the beds. Owing to the prolonged period over which both the first and second generation of flies may emerge, the correlation that exists between sowing date and subsequent age of the crop in its attractiveness to the gravid females, and the date of emergence of the summer generation makes necessary extremely careful timing in the application of poison-bait mixtures.

The peak period of emergence of first generation flies at Wisley varies from 8–18 May, being dependent upon climatic conditions. As the emergence of flies in the laboratory precedes that in the field by some 10–12 days, there is adequate time to forewarn growers as to the time to prepare for baiting. A further plea is made, therefore, for the preparation of 'Standard Emergence Tables' for various carrot-growing areas as outlined by Barnes (1942). The dates of appearance of carrot flies in various localities from the standpoint of timing the application of baits is of paramount importance, and requires the co-operation of Advisory Entomologists throughout the country. With such phenological data available, the applied biologist would be able to convince the grower that correct timing in the application of poison-bait mixtures is essential.

The hedges adjoining one carrot bed were chosen for baiting, while another bed with the same aspect was left untreated. Both beds were sheltered from the north, east and west by hedges in which the flies assembled. The poison mixture used was that recommended by Petherbridge *et al.* (1942) and Petherbridge & Wright (1943), and was applied with the aid of a continuous-spraying syringe to the hornbeam foliage on the following occasions: (a) against first generation flies: 20, 24, 27, 31 May; 3, 7, 10, 19, 23, 29 June, (b) against second generation flies: 10, 12, 15, 17, 19, 30 Aug.; 1, 9 Sept. (dates in italics after singling).

The applications were for choice made on warm, overcast days with little or no wind when the flies were active though remaining in the shelter of the hedges. It was found that during low temperatures and high wind, the conditions are unsuitable for activity as then the flies remained sluggish and made little or no attempt to feed. A comparison between the percentage of unsaleable roots from the bed around which the hedges were sprayed and those from the unsprayed bed is shown in Fig. 6.

While the application of a poison bait has been shown to be both practicable and effective under commercial conditions (Petherbridge *et al.* 1942; Petherbridge & Wright, 1943), this control measure has serious disadvantages with garden crops. The chief reasons against its general adoption in gardens and allotment-areas are: (i) the number of applications that are required to effect a marked reduction in first generation fly populations—the amateur being loath to make a number of applications at any time against any one pest and is especially critical of any measure that requires exact timing; (ii) the injury that follows the application of this bait to hedge plants—severe leaf scorch followed its application to hornbeam foliage at Wisley; and (iii) the cancelling out of its effectiveness where there exists alternative shelter sites for the flies, e.g. nettle beds, rough herbage and adjacent plots of potatoes. Such factors are sufficient to render this method of control applicable only to field crops.

The crop of annual nettles that grew in close proximity to the carrot bed at Wisley this year had the effect of considerably reducing the effectiveness of the bait (Fig. 6).

Other chemical methods of control have been investigated by various workers, including the application of 4% calomel dust, 'spent' soot, naphthalene and the stretching of creosoted twine some inches above the rows of plants, all of which measures serve merely as repellent agents against the egg-laying females. The use of creosoted twine, however, deserves mention owing to its suitability in gardens and allotments. This was clearly demonstrated in some London parks in 1943—a year of severe carrot-fly attacks. Those allotment-holders who had adopted this method were able to lift a comparatively clean crop of roots, while crops on neighbouring allotments which were not protected in this way were rendered useless for human consumption.

Varietal susceptibility to attack. Petherbridge & Wright (1943) stated that the number of eggs laid on young carrots appears to increase with the size of the tops or with some other factor which increases with age. In this connexion, there appeared in the 1942 trials to be some evidence of varietal susceptibility to attack in the vars. Early Market and Early Nantes (Fig. 2). It was considered probable that if any difference did occur, the factor influencing the degree of attack was the density of the foliage of the

young plants in relation to the size of the roots. This apparent difference in varietal susceptibility to attack occurred to some degree in 1943-4 (Table 1).

TABLE 1

Variety	Density of foliage in singlings	Cleaf roots %	Damaged roots %
Danvers	Moderately dense	10.0	90.0
Early Market	Very dense	10.5	89.5
Early Nantes	Normal	9.0	91.0
James's Scarlet	Very dense	8.8	91.2
Intermediate			
New Early Scarlet	Normal	15.0	85.0
Horn			

While there appears to be no evidence that there exists any correlation between density of foliage in the young plants and the degree of attack, the number of eggs laid near carrots does increase with the size of the foliage irrespective of varietal differences in

density. The Wisley observations confirm those of other workers in that there is little varietal difference in the degree of infestation. Petherbridge *et al.* (1942) found that on all sowings of three varieties of carrot there was no significant difference.

Investigations such as those outlined are possible only with the co-operation of a number of workers, and it is my pleasant duty to acknowledge with grateful thanks the help received from my Assistant, Miss P. S. Shilton, who helped with the sowings and in washing, grading and weighing some 30,000 roots during 1942-44, and who has been entirely responsible for the preparation of the charts and graphs outlining the results; from my colleague, Mr F. C. Brown, for constant advice on matters relating to varieties, sowing dates and general cultivation; and from the Misses Field, Clegg-Thomas and Rhodes for carrying out the major portion of the work of cultivation during the three seasons.

REFERENCES

- BAKER, F. T., KETTERINGHAM, I. E., BRAY, S. P. V. & WHITE, J. H. (1942). Observations on the biology of the carrot fly, etc. *Ann. appl. Biol.* **29**, 115.
- BARNES, H. F. (1942). Studies of fluctuations in insect populations. IX. The carrot fly (*Psila rosae*) in 1936-41. *J. anim. Ecol.* **11**, 69.
- FOX WILSON, G. (1943). A note on the control of the onion fly (*Delia antiqua*). *J. roy. hort. Soc.* **68**, 276.
- PETHERBRIDGE, F. R., WRIGHT, D. W. & DAVIES, P. G. (1942). Investigations on the biology and control of the carrot fly. *Ann. appl. Biol.* **29**, 380.
- PETHERBRIDGE, F. R. & WRIGHT, D. W. (1943). Further investigations on the biology and control of the carrot fly. *Ann. appl. Biol.* **30**, 348.

(Received 8 December 1944)

Proceedings of the Association of Applied Biologists

Joint Meeting of the Association of Applied Biologists and of the Genetical Society held on Friday, 23 March 1945 in the London School of Hygiene and Tropical Medicine. Chairman at the morning session: Dr C. B. Williams, President of the Association. Chairman at the afternoon session: Dr C. D. Darlington, President of the Genetical Society.

Symposium on the genetical relations of plants and animals to their pests and diseases

Morning session

- I. Introduction: the genetic analysis of disease. By C. D. DARLINGTON.
- II. 'Constitution' in cattle in relation to pests and diseases. By J. HAMMOND.
- III. Observations on heritable disease in poultry. By M. S. PEASE.
- IV. Heritable susceptibility of poultry to cancer virus. By J. G. CARR.

Afternoon session

- V. Inheritance of resistance to blight in potatoes. By W. BLACK.
- VI. Some genetical aspects of resistance to potato viruses. By G. COCKERHAM.
- VII. Diseases and pests at the Welsh Plant Breeding Station, Aberystwyth. By T. J. JENKIN.
- VIII. General discussion and summing up. By C. B. WILLIAMS.

Introduction: the genetic analysis of disease

By C. D. DARLINGTON, *John Innes Horticultural Institution, Merton, London*

Two laws govern the genetics of disease. The first is that all variable species, from man to the bacteria, vary genetically in power and mode of defence against all the diseases that attack them. The second is that, on their side, all variable disease organisms vary in power and mode of attack on their victims and also of defence against our chemical and biological attacks. The result is that when we change the situation by introducing a new pest-resistant plant or animal host, or a new pest-killing agent, whether it is cyanide or sulphonamide or penicillin, we are applying selection to which the pest, scale insect, *Gonococcus*, rust or virus, reacts by producing new forms with suitable new powers of attack or defence. The interactions of bacteria and bacteriophages illustrate diagrammatically this struggle between mutation and counter-mutation.

For long-term policy we therefore have to examine and compare the system of breeding and variation, the means of adaptation of both the host and the pest. In nature variability is the rule and fixity the exception for both. But crop and stock improvement of the last 50 years has ruined this natural balance. It has imposed close kinship and fixity of an ever-

diminishing number of ever-extending types of crop or stock which give the pest or disease an ever-easier kill. The extremity is reached with the relationship between a dense population of an absolutely uniform victim, the vegetatively propagated plant clone—potato, strawberry, raspberry or banana—and the highly mutable and adaptable virus. The genetic remedy is the only permanent one for this genetically untenable position. It is to introduce new variation into the cultivated forms and, as Crane has suggested, to restore a system of sexual reproduction by the regular production and replacement of short-lived clones.

Viruses are of interest in genetics from other points of view. Their analogies with nuclear genes and with cytoplasmic heredity in mutation and in their effects on variegation and the production of specific antibodies are obviously significant. J. M. Wallace has shown (*J. agric. Res.* 1944, **69**, 187) that antibodies to a virus are produced by tobacco and that they can propagate themselves indefinitely in a tomato which cannot develop them itself. These antibodies are conveyed by grafting, but not by the virus vector. There is nothing surprising in this since only the

virus has been selected for transmission. But it makes possible the separation of the virus from the antibody.

Thus self-propagating bodies, presumably nucleoproteins, created by one organism can propagate themselves in an entirely different organism. They can be transmitted by grafting, but not always by a

vector. This argument reinforces the evidence of the origin of viruses themselves by grafting adduced by Johnson, Salaman & Le Pelley, and Crane. It suggests that a genetic and evolutionary treatment of the problems of virus as well as other diseases can help in planning their solution.

'Constitution' in cattle in relation to pests and diseases

By J. HAMMOND, *School of Agriculture, Cambridge*

The genetic relation of animals to their pests and diseases can best be illustrated by what happens when European cattle are introduced into the tropics. There they are extremely susceptible to local pests and diseases to which the native or other tropical cattle are comparatively resistant.

European cattle in the tropics have a higher incidence of tick infestation than the Zebu, which is probably due partly to their longer hair (Kelly, 1943) and to their higher body temperature. There is considerable variation in the length of hair in European cattle in the tropics (Bonsma, 1943) and there is also some evidence of segregation of this character in European-Zebu crosses (Hammond, 1932).

The degree of tick infestation regulates the intensity of infection by the piroplasmosis of tick fever. In Zebu cattle there is no evidence for any immunity to infection. The main difference between European and Zebu cattle lies in the fact that the resistance to the effects of infection is very much higher in Zebu than in European cattle (Kelly, 1943); first crosses come nearer to the Zebu resistance (Frona, 1930).

An explanation of these facts is that the nutritive state of the body depends on the power of heat regulation of the animal: where this is defective and a low nutritive state exists the power of resistance to infection by piroplasmosis is low. Rhoad (1941, 1942)

has measured the body temperature and dissipation of energy, as shown by increased respiration rate, in crosses and backcrosses between the Zebu and European cattle. The milk production and growth of these crosses and backcrosses run parallel with their resistance to tick fever and there can be little doubt that they are all due to differences in physiological constitution mainly connected with the heat regulation powers of the animal at high air temperatures. For tropical conditions new breeds—the Santa Gertrudis (Rhoad, 1941); the Philamin (Frona, 1930, Manresa, 1934)—are being formed to combine the productive powers of European beef cattle with the heat, tick and disease-resisting qualities of the Zebu.

Little experimental work on the 'physiological constitution' of cattle has been done in this country and only speculations can be made on the basis of practical experience. There is some experience to show that genetically high milk producing cows have reduced resistance to diseases when nutrition conditions are poor. The intensity of competition of the udder with the other tissues of the body for incoming nutrients is determined by genetics and this in turn determines resistance to disease at low nutritive levels, although not necessarily at high nutritive levels.

REFERENCES

- BONSMAN, J. C. (1943). *Fmg S. Afr.* Feb.
 FRONDA, F. M. (1930). *Lingnan Sci.* 9, no. 3, 215.
 HAMMOND, J. (1932). *E.M.B. [Publ.]*, 58, Nov.
 KELLY, R. B. (1943). *Bull. Coun. sci. industr. Res. Aust.* p. 172.
 MANRESA, M. (1934). *Philipp. Agric.* 23, no. 5, 433.
 RHOAD, A. D. (1941). *Yearb. U.S. Dep. Agric.*, 508.
 — (1942). 8th Amer. Sci. Congr., Biol. Sect., Gen. Biol. p. 115.

Observations on heritable disease in poultry

By M. S. PEASE, *School of Agriculture, Cambridge*

Previous workers have claimed that a flock of poultry resistant to fowl paralysis could be built up by selection; and the virtual disappearance of fowl paralysis in recent years has been attributed to selective breeding. The disease was first noticed in the Cambridge flock in 1930, the incidence rising to an alarming peak in 1935 and thereafter declining to negligible proportions in the war years. Records from laying trials throughout the country showed that the experience at Cambridge was typical of that in the country generally. An analysis of the incidence of this disease in the Cambridge flock year by year disclosed no evidence whatever that inheritance played any part in its causation.

With regard to non-specific disease in poultry, it was widely held that this was due to persistent breeding from pullets, that 'immature' breeders produced weakly offspring. A statistical analysis of chick and adult mortality in the Cambridge experimental flock showed that the determining factor was not the age of the mother, but whether the mother

lived or died during the breeding season. Mothers which died left progeny with an excessive death-rate: mothers which lived, whether pullets or older birds, alike left progeny with a lower death-rate. Statistics showed that the death of the sire during the breeding season was without effect on the viability of the progeny. Evidence was against attributing this to the action of Y-borne genes. It was therefore concluded that the quality of the egg cytoplasm must be in some way affected, either by the deficiency of essential constituents or by the presence of toxic substances from the ailing mother. An analysis of mortality in relation to the position of the egg in the mother's laying cycle and also to its position in the clutch lent some support to this view. The chicks hatched from eggs laid early in the cycle had a better viability than had those from eggs laid later in the cycle—and the same was found to apply to the individual clutches. It would seem, therefore, that eggs laid immediately after a pause are better provisioned for the developing embryo.

Heritable susceptibility of poultry to cancer virus

By J. G. CARR, *Institute of Animal Genetics, Edinburgh*

There is a very high incidence of cancer in the domestic fowl of all breeds in every part of the world. It is usually considered that the majority of these infections are due to the action of carcinogenic viruses, of which a number are known.

Using the Rous No. 1 sarcoma as a test virus, and a 6-8-weeks-old chick as host, it was possible to show that the main factor in determining the extent of tumour growth was the genetic constitution of the host, and other factors were not involved to any great extent. Results obtained with the Brown Leghorn flock of Dr Greenwood are presented. From the data thus obtained, it was found possible

to produce by selection a line of fowls exhibiting a very high resistance to the tumour virus. This resistance was not due to any transfer of inhibiting material through the egg, as indicated by breeding and other experiments. It was found that this line, though bred for resistance only to the Rous No. 1 virus, was also resistant to other carcinogenic viruses, and to a certain extent to chemically induced tumours.

The mode of transmission of the virus among fowls remains unknown. Effective transmission by the egg does not occur, and contact of normal birds with virus-infected fowls does not seem to cause infection of the normal fowls under conditions so far tried.

Inheritance of resistance to blight in potatoes

By W. BLACK, *Scottish Plant Breeding Station, Edinburgh*

In the search for new potato varieties possessing resistance to blight (*Phytophthora infestans*), the wild polyploid species, *Solanum demissum*, was employed as the source of resistance genes. The appearance of biotypes of the parasite has rendered the task more complex.

Three strains of blight were isolated and used for

test purposes, viz. strain A, the common one found in commercial crops, and strains B and C which appeared on plants known to be immune from strain A. By testing a mixed collection of hybrids with these three strains, four different resistant phenotypes were differentiated, viz.:

(1) Plants immune from A, B and C.

(2) Plants immune from A and B, but susceptible to C.

(3) Plants immune from A and C, but susceptible to B.

(4) Plants immune from A, but susceptible to B and C.

Since all the resistant plants were, in the first place, immune from strain A, this strain appears to be less virulent than strains B and C. The difference in virulence between strains B and C, however, was found to be qualitative rather than quantitative and they must be regarded as physiologic forms adapted to suit particular hereditary types of host plants.

Parallel tests of derivatives of three species, *S. Rybinii*, *S. demissum* and *S. tuberosum*, were made with strains A and B. The ratios of immunes to susceptibles resulting from infection with strain A were 1:1, 3:1 and 7:1 in three series of backcross progenies, and 3:1 and 63:1 in two series of selfed progenies. The corresponding ratios resulting from infection with the B strain were 0:∞, 1:1 and 3:1 for backcrosses and 0:∞ and 15:1 for selfed progenies. Further, the self-fertilization of A immunes failed to

produce B immunes. It is therefore concluded that immunity from the A and B strains of blight is controlled by two major genes **Ra** and **Rb**. Plants carrying the **Ra** gene are immune from the A strain alone, while plants carrying the **Rb** gene are immune from both strains. Minor genes which merely control the degree of susceptibility in susceptible varieties and act as unidentifiable modifiers in resistants may also be assumed.

Three groups of progenies, involving over 18,000 plants gave segregations which were characterized by an excess of susceptible seedlings. The extent of the deviations from standard ratios was greater than that attributable to double reduction in autotetraploids. Since some degree of incompatibility exists between *S. demissum* on the one hand and *S. Rybinii* and *S. tuberosum* on the other, it seems probable that the excess of recessives was largely due to differential compatibility of the gametes. Blight resistance is essentially a *demissum* character and so long as it is retained so also may be some residual incompatibility to upset the balance of segregation.

Some genetical aspects of resistance to potato viruses

By G. COCKERHAM, *Scottish Plant Breeding Station, Edinburgh*

Hypersensitiveness to and hence field immunity from a number of potato viruses and virus strains is conditioned by the genetic constitution of the potato host. Hypersensitive response to several strains of virus *X* occurs in the presence of a single dominant gene, **Nx**. This gene is ineffective, however, against a number of aberrant strains of the virus. Hypersensitiveness to some of these strains is nevertheless under genetic control and there are grounds, as yet largely circumstantial, for postulating a related series of genes as the determinants. One of these latter genes, that controlling the hypersensitive response to virus *X^B*, has already been investigated and designated **Nb**.

The gene **Na**, determining hypersensitiveness to virus *A*, is closely linked to **Nx** in each of six cultivated varieties. The linkage is in the coupling phase in each variety and no linkage in the repulsion phase has yet been detected. In the variety Southesk, however, the two genes show independent and tetrasomic segregations.

Hypersensitiveness to virus *C*, a strain of virus *Y*, is determined by a gene **Nc**. Varieties carrying this gene in the simplex condition manifest strikingly similar necrotic symptoms during the early stages of infection with both virus *C* and virus *Y* but, whereas infection with virus *C* passes rapidly into a lethal disease which entirely destroys the affected plant,

infection with virus *Y* leads to a non-lethal streak and ultimately to a mosaic disease. Varieties carrying the recessive alleles, **nc**, usually respond to both strains of the virus by manifesting anecrotic symptoms of similar mosaic diseases. This correlation suggests that the gene **Nc** conditions not only the hypersensitive response to virus *C*, but also the non-lethal streak reaction to virus *Y*.

Although no cultivated variety of the potato is hypersensitive to virus *Y*, five clones of three different 'wild' species show the necrotic responses characteristic of hypersensitiveness when they are infected with the virus in the laboratory. Indication has been obtained that the ability to respond in this fashion is inherited as a unit character, but the available information is yet insufficient for full genetic interpretation.

A general consideration of field immunity through hypersensitiveness leads to the view that the character is of significant value in virus control. The special advantages attached to it are that the genes concerned are readily available, most of them within cultivated varieties, and there is no evidence of adverse linkages with undesirable characters. The four genes, **Nx**, **Nb**, **Na** and **Nc**, have been brought together in commercially acceptable varieties. A potential disadvantage arises in the inherent variability of individual viruses since there is the possibility that

hypersensitive varieties may exercise a selective effect in favour of aberrant virus strains from which they are not field immune. Indeed, aberrant strains have been found already in field immune varieties, but only as exceptional occurrences. There is no indication of their widespread dissemination within such varieties, even in those which have been extensively cultivated for over 40 years.

A high degree of positive resistance to the leaf roll virus is found in three potato varieties, two of which

are known to have 'wild' forms in their ancestry. On the evidence of the comparative behaviour of named varieties and of seedling progenies in diallel series, and from the segregations observed in individual families of selfed and hybrid parentage, it is suggested that the resistance is heritable and under genic control. Several factors appear to be concerned, and there is indication that though they only delay infection when dispersed they may entirely prevent infection when sufficiently aggregated.

Diseases and pests at the Welsh Plant Breeding Station, Aberystwyth

By T. J. JENKIN, *Welsh Plant Breeding Station, Aberystwyth*

The Station is mainly concerned with the production of improved varieties or strains of oats, clovers, and herbage grasses. The capacity to resist the attack of any particular disease or pest is one desirable attribute of a new strain, but there are other attributes that may be equally important. In practice, the incidence of diseases and pests is noted, and observations are often made. In a few cases the inheritance of resistance and susceptibility has been studied in some detail, but no attempt has yet been made to study biologic races of the parasite.

In oats, the choice of breeding material has been limited by the virtual disappearance of old 'land' varieties composed of an intimate mixture of sub-varieties in the production of which natural inter-crossing may have taken some part. By selection from mixed populations of *Avena strigosa* a variety, S. 75, which is highly resistant to smuts has been produced, while in S. 171 this has further been combined with a relatively awnless grain type. The Station-bred white winter oat variety S. 81 is one of the varieties now in cultivation most resistant to eelworm.

In the clovers, special attention has been paid to clover rot (*Sclerotinia trifoliorum*) and clover sick-

ness (*Tylenchus devastatrix*), but again the aim is to produce resistant strains rather than to study the disease or pest in detail. The choice of breeding material in clovers is far wider than in oats owing to the heterogeneity of the crops and the heterozygosity of individual plants.

This is still more marked in the herbage grasses, where there is abundant wild material from which selection can be made. Observations suggest that the study of the inheritance of resistance and susceptibility may not be easy owing to (1) the complexity of the material, with variation from plant to plant and from family to family, (2) the absence of attack in different areas and seasons, (3) the association of resistance or susceptibility with age or stage of development of the individual plants, (4) biologic races, and so on. In practice, plants that are themselves obviously susceptible are rejected as breeding material, as also, generally, are parent plants whose progeny have shown lack of resistance. Blind seed disease of perennial ryegrass and the cocksfoot moth may prove to be serious in relation to strain multiplication and commercial seed production. A condition resembling virus disease has been encountered in old breeding material of perennial ryegrass.

General discussion and summing up

By C. B. WILLIAMS, *Rothamsted Experimental Station,*
President of the Association of Applied Biologists

In the discussion which followed the principal contributions, Prof. W. J. B. RIDDELL called attention to the effect of the different varieties of *Pneumococcus* causing pneumonia. In Glasgow, type II occurred with higher frequency than in Manchester, where type I was the most common. Since the introduction of chemotherapy type II had relatively increased in Glasgow.

K. MATHER mentioned the work of Lindstrom and his pupils on the bacterial wilt of maize. In this case the bacterium increases in virulence by passage through a resistant inbred line of maize, but decreases in virulence by passage through a susceptible line. In mixed inoculations the virulent strain is selectively favoured by resistant maize and the avirulent by susceptible lines.

M. B. CRANE discussed the effect of the use of 'clonal' crops and suggested that some of the dangers might be avoided by the regular production of short-lived clones or by the regular production of F_1 families which are sufficiently uniform for commercial requirements. Virus infection upsets uniformity, even in clonal crops. In some vegetatively produced plants the position has now been reached when as much care, or more, has to be given to the nursing of old clones as to their cultivation.

F. C. BAWDEN did not agree that present available evidence proved the existence of virus antibodies in plants.

H. W. HOWARD said that at Cambridge experiments had been carried out to obtain potatoes resistant to blight, using *S. demissum* as the source of immunity, with results very similar to those obtained by Dr Black at Edinburgh. In F_6 and F_7 , however, there was some indication of the effect of modifying genes being of importance. He considered that the genetics of the parasite (*Phytophthora infestans*) needed more investigation. Strains from single

spores had not changed their virulence and the origin of new strains may be due to sexual reproduction.

R. STENTON suggested that the ratio of diseased individuals in a mixed population of unknown genetic constitution might be found to be proportional to the number of recessives present.

C. B. WILLIAMS (President of the Association of Applied Biologists), in summing up, re-emphasized the importance of keeping a high level of variability in the host plants and animals as a protection against possible new diseases in the future. He gave a short account of work recently carried out by Dr S. C. Harland in Peru on the selection of cotton. Harland started with a very large number of plants tested individually for a small number of commercially valuable characters, and selected for these only, leaving all other characters as variable as possible. He avoided all self-fertilization, which produced a large proportion of homozygotes for all characters both visible and invisible.

Reviews

Microbial Antagonisms and Antibiotic Substances. By SELMAN A. WAKSMAN. Pp. ix + 350. New York: the Commonwealth Fund (London: Oxford University Press). 1945. \$3.75 (21s. 6d.).

Dr S. A. Waksman, Professor of Microbiology in Rutgers University, U.S.A., has long been acknowledged as the leading investigator in the field of soil microbiology. Beginning some 30 years ago with simple papers entitled 'Do fungi actually live in the soil and produce mycelium?' and 'Is there any fungus flora of the soil?' he has, in the interim, added more voluminously to our knowledge of soil organisms and their role in the soil economy than any other worker. Original studies by Waksman, his collaborators and students, have largely filled the journal *Soil Science*; in 1927 appeared his masterly 900-page volume on the *Principles of Soil Microbiology*, with a second edition 4 years later; in 1931 he and Prof. Starkey produced *The Soil and the Microbe*—still the best introduction to soil microbiology; and in 1936 he published a 500-page volume on *Humus*, which appeared in a second edition 2 years later. Much of Prof. Waksman's earlier work was systematic and descriptive, but he soon began also to study the biological relationships of the soil organisms and their physiological and biochemical characteristics. Throughout the whole of his work he has visualized the soil population not as an agglomeration of so many individual organisms living independent lives but as a functional organic complex, a world in microcosm, in which organisms have place and value in terms of their biological relationships, their mutual activities and their antagonistic and associative interactions. He was, therefore, peculiarly well equipped in mental outlook, knowledge and experience when, in the mid-1930's, he turned his attention to the newly opened field of antibiotic substances produced by soil and other micro-organisms. During this decade papers on the subject, both original investigations and more general surveys, have flooded from his pen, and he has now added to our indebtedness by writing this fine volume.

The study of the antagonistic relationships of micro-organisms presents some of the most obscure and difficult problems which confront biologists to-day. Their solution has been helped in certain ways and hindered in others by the fact that the subject has been developed with phenomenal rapidity and largely to specific practical ends, and primarily by teams of workers operating intensively within narrowly restricted and disparate fields of inquiry. But this is a subject which transcends the specialized compartments of science. The physiology and microbiology of the soil, mycological and bacterial systematics, the morphology, physiology and genetics of micro-organisms, biochemistry and pharmacology, plant, animal and human physiology and pathology, veterinary and clinical medicine all meet and integrate in this newly exploited field of science, so that an advance in any one direction reacts immediately and fundamentally in many other directions. For the investigation of any particular problem within this wider field specialized knowledge and technical expertise are essential, and the narrow specialist plays his part in advancing the detail of knowledge. But, if the subject is to advance on a broad and even front and develop a coherent theory weaving the particular into the general to

form a comprehensive fabric of science, the particular issues must be seen not as so many autonomous problems having empirical solutions but as a correlated body of logical relationships in a common perspective. Prof. Waksman has attempted just this; and the great value of his book lies not so much in the mass of factual knowledge it contains as in the marshalling of principles and data into a logical whole which shows clearly the historical development of the subject and the natural interrelationships of its parts. Only a student of Prof. Waksman's unrivalled knowledge and experience of microbiological problems could have made this intellectual synthesis. It would have been easy for any one of a number of workers in this obscure and difficult field to write a volume on *Penicillin*, on *The Chemical Nature of Antibiotic Substances*, or on any other special issue, but the hallmark of Prof. Waksman's treatment is his appreciation of the fact that the particular problems and issues are only special cases in the general perspective of microbial association and antagonism.

It is thought by many people that the concept of microbial antagonism originated in Prof. Fleming's observations on *Penicillium notatum* in 1929: this is not the case, for it has had a long and interesting history dating back to Pasteur and deriving from various fields of microbiological study. The limelight of war-time necessity, concentrated mainly on penicillin, has given this substance perhaps undue pre-eminence in both the popular and the scientific imagination. Penicillin is, however, merely one of many similar substances produced by micro-organisms, although, in its particular chemotherapeutic properties, it still seems to be of outstanding importance among them. To say that the concept of microbial antagonism was familiar ground long before 1929 is not in any way to denigrate the originality or the vast potential importance of Prof. Fleming's observations or the brilliant exploitation of them by Prof. Florey and others which has led to such magnificent practical achievements in the field of disease control. It is to see all this work in its proper setting as the opening of a new and exciting chapter in a book of which the earlier chapters sketched in the historical and relational background. The really interesting question is why penicillin and other antibiotic substances were not discovered and applied much earlier. Looking back over the development of the concept of microbial antagonism one cannot help feeling that the state of microbiological science was such that the time was ripe for the discovery at least a decade before it happened, and that all of us who were working in this field were astonishingly blind to values that were literally staring us in the face. The phenomena were commonplace to bacteriologists, mycologists, plant pathologists and soil microbiologists in their daily laboratory routines, but they entirely failed to draw the obvious conclusions from them or to realize their implications. The belated nature of the discovery and of its application is probably also largely due to the compartmenting of science which so unfortunately and for so long separated mycology, plant pathology and soil microbiology from medical bacteriology, human pathology and clinical medicine. It is the recognition of the evils of this separation of the medical, the industrial, and the 'scientific' aspects of microbiology which has led to the recent founding in this country of the *Society for General Microbiology*, a most welcome development

which should go far to ensure cross-fertilization between these branches and a more rapid and even progress in this vastly important field of scientific inquiry and biological application.

But to return to Prof. Waksman's book which has been the occasion for these digressions. The author first surveys the broad interrelationships among micro-organisms living in association, either in simple mixed cultures as in laboratory experiments or in complex natural populations as in sewage, soil, water, etc., special attention being paid to the antagonistic effects. Emphasis is laid upon the significance of these associations in natural processes and upon their relation to disease production in man and in his domesticated animals and cultivated plants. The methods of isolation and cultivation of antagonistic micro-organisms are described as well as the techniques for measuring antibiotic action. There are chapters dealing respectively with bacteria, actinomycetes, fungi, and microscopic animal forms as antagonists, and with the antagonistic relationships between micro-organisms, viruses and other non-specific pathogenic forms. The chemical nature, so far as it is known, of the antibiotic substances produced by various micro-organisms is described. The nature of the antagonistic action and its utilization for practical purposes of disease control are discussed, a chapter being devoted to the microbiological control of soil-borne plant diseases. Finally, the author puts forward some interesting speculations on the outlook before us regarding these fascinating problems. An appendix—already out of date, so fast is this subject developing—contains a classification of antibiotic substances. The fact that one organism may produce more than one antibiotic substance and that the same substance may be produced by more than one organism has led to difficulties of nomenclature and synonymy, but there would seem to be known seven antibiotic substances produced by actinomycetes, one by algae, eleven by bacteria, and fifteen by fungi. To the latter at least one more—viridin produced by *Trichoderma viride*—must now be added, and there is not the slightest doubt that the list in each section of the classification will rapidly increase. There is a short glossary, a splendid bibliography (already incomplete) of 1037 references, an index of micro-organisms, and a general index. The book is illustrated by twelve excellent plates and numerous text-figures and is beautifully produced.

So much attention has been concentrated on penicillin and its application in clinical medicine, as was imperative in war-time, that plant and animal pathologists have tended to overlook the potential importance of antibiotic substances in their own fields of endeavour. Such workers will find this book of unusual interest. Although it is a technical survey it is yet unexpectedly readable, and it seems to me quite the most stimulating and suggestive publication on this subject that has come my way.

WILLIAM B. BRIERLEY

Root Disease Fungi. By S. D. GARRETT. Pp. 177. Waltham, Mass., U.S.A.: the Chronica Botanica Co. (London: Wm. Dawson and Sons, Ltd.). 1944. \$4.50.

Mr Garrett was once described to me as 'a dogged sort of worker', and he has written a dogged sort of book. Scattered diffusely through the world's scientific journals there is a mass of information on the root-disease fungi and the troubles they cause, and Mr Garrett has waded pertinaciously through this material, sifting out the grain from the chaff. He has organized his findings in the

light of his rich personal experience of these problems, has drawn definite (perhaps over-definite!) conclusions and has formulated certain general principles which he has applied to the control of root diseases. Out of the originally inchoate material he has fashioned an orderly and logical edifice which, of its kind, is a model of constructive work.

The contents of the book group themselves into four progressive themes. Chapters 1-3 discuss the microbiological equilibrium of the soil and the biological relationships of the fungi classified as soil inhabitants, soil invaders, and mycorrhiza. Chapters 4-6 deal with the influence of the physical and chemical factors of the soil upon parasitic activity. Chapters 7 and 8 consider the saprophytic life and dormancy of root-infecting fungi. Finally, in the light of the data presented and the conclusions drawn from them, the remaining seven chapters are devoted to the principles of root-disease control and their application to crops grown under field, plantation, and glasshouse conditions. The book concludes with a bibliography of some 450 references, a general index and an author index.

Mr Garrett disclaims any intention of dealing comprehensively either with all root-infecting fungi, or with the diseases they cause; he focuses his attention on a comparatively small number of well-known fungi which cause diseases of notable economic importance. Nevertheless, the fact that the root-infecting fungi exist as an ecological complex in what is a comparatively stable environment, means that his conclusions and the principles he formulates have comprehensive application. To have made a complete survey of all the relevant fungi and diseases would merely have increased the number of confirmatory examples without adding to the cogency of the author's analysis. Actually, his survey is sufficiently complete to justify thoroughly the somewhat quaintly worded subtitle of the book which reads 'A Treatise on the Epidemiology of Soil-borne Diseases of Crop Plants, and a First Exposition of the Principles of Root Disease Control'.

The book is so tightly packed with data and points for discussion that to consider it in detail, as one would like to do, would entail writing a volume of equal length. But I would like to enter one caveat against the author's general treatment of his subject which is that, in my opinion, he seriously underrates the capacity of many facultative parasites to live saprophytically in the soil for long periods and, perhaps, indefinitely. This underestimation would seem to result from what are, again in my opinion, two doubtful viewpoints. The first is that it is safe to reason directly from small-scale and simple laboratory experiments to what may happen under the infinite variety of crop and soil conditions in the field. In my own experience of soil and disease problems this is a thoroughly unsafe procedure. The second is that there is any sharp cleavage between soil invaders and soil inhabitants. To my mind these terms merely define the end-points in a relatively unstable transitional series in which the place of any given fungus at any given time is conditioned partly by the nature of the organism and partly by the locally fluctuating soil factors. I cannot help feeling that Mr Garrett's dogged mind has led him to underestimate the adaptability of fungi and to oversimplify the vast complexity of the situation as it occurs in nature.

No one, however, will look this gift horse too closely in the mouth; it is an uncommonly well-groomed and useful animal and a real credit to its producer.

WILLIAM B. BRIERLEY